THE RELATIONSHIP OF SORE THROATS WITH VIRAL INFECTIONS.

by

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PART I.

INTRODUCTION.

The scope and purpose of scientific research keeps on changing as more knowledge is gathered about specific problems. There was a time when workers had only tackled the problems of major diseases which had been known to mankind, since the beginning of history; the minor illnesses such as respiratory disease were considered as the integral part of human living experience. As time passed and the basic facts about the major diseases were discovered more attention was paid to the minor problems.

Although signs of a different approach to the research into the virology of upper respiratory tract infections had been in the air for a few years, (Commission on acute respiratory diseases 1946, Dingle 1948, Stuart-Harris 1953, Brimblecombe et al 1958), the change itself did not occur until after the Conference on newer respiratory disease viruses in October 1962 (Amer Rev Resp. Dis. 1963). Since then the emphasis has shifted from the concept of one virus being isolated from one syndrome to finding out the variety of viruses capable of being associated with a particular set of symptoms.

Immediately after the conference Higgins and associates in 1963 published their first work on the isolation of viruses from acute respiratory infections occurring in general practice and followed it up with other work /

work in the same field (Higgins et al., 1963, 1964, 1966 , Higgins 1966a, 1966b). Since Higgins' first publication, other studies have also been conducted in this country in the same or closely related fields (Tobin 1963, Clarke et al 1964, Holzel et al 1965, Banatvala et al 1965), and in the U.S. and Australia (Parrott 1963, Wulff et al 1964a, Adams 1965).

The interest of British workers in the study of acute respiratory infections in the general population resulted in the publication of a collaborative study of the aetiology of acute respiratory infections in Britain 1961-64 by a working party of the Medical Research Council of Great Britain 1965. These studies, having been conducted on a country wide scale by a large number of workers, encompassed a variety of upper respiratory illnesses (common cold, feverish cold, influenza, sore throat, pharyngitis, tonsillitis, croup, tracheitis etc., bronchitis, bronchiolitis, bronchopneumonia, others), each one of which in our view required full time interest from a separate group of workers.

With the background of the aforementioned knowledge (M.R.C. Report 1965), it was decided to carry out an investigation into the aetiology and epidemiology of the sore throat syndrome in a general practice.

The position taken in this study has been that at the /

the present stage of our knowledge we have to be content with the idea that respiratory tract infections represent a mixture of diseases caused by a mixture of many different viruses. (Tyrrell, 1963. Parrott et al 1963, Dowling and Lefkowitz 1963).

In an attempt to solve any aetiological problem various symptoms associated with a particular syndrome must be recorded and correlated with the virus isolation results. For this study sore throat has been used as the indicator symptom and attempts have been made to correlate the pattern of symptoms with the result of isolation of respiratory viruses.

REVIEW OF LITERATURE.

Clinical terms used in upper respiratory tract infections.

In upper respiratory disease there are many vague clinical terms which required careful assessment. In order to evaluate these terms it is necessary to study their evolution. These clinical manifestations are definite stages in the biological evolution of host parasite relationships and are the result of the reaction of the tissues to the invasion by a parasite or the action of an irritant; their magnitude and severity depends upon how severely the tissues are damaged and on how great is their reaction.

The reaction of the upper respiratory tract is so limited that the response seems to be similar whatever the cause. For example inflammation of the nasal mucosa makes it hyper-irritable resulting in sneezing, mucous discharge, blockage of air passages and catarrh. This is the limit of the response of the nose to acute inflammation and the nature of the causal agent, be it a virus, or an allergen, has little effect on the symptoms. Similarly the response of the larynx is limited to a sore dry tickling sensation, an irritating cough and loss of voice or hoarseness due to oedema and hyperaemia of the vocal cords.

The sites of these reactions gave rise to anatomical definitions such as rhinitis, pharyngitis, laryngitis /

laryngitis and tracheitis without giving any hint of the aetiology of the condition. Historically, the expression of clinical symptoms in terms of anatomical sites thus became an accepted practice. Although these are not based on the bacteriological or virological aetiology of the disease, they have proved very useful in providing incentives for the search for aetiological agents.

The terms that are of importance to us were used by physicians long before the advent of bacteriology and were taken over by bacteriologists to form the basis of their aetiological studies. Apart from the syndromes for which a bacteriological aetiology was established or which were so markedly distinct as to give them separate entities, other symptoms were put together under simple headings such as upper respiratory tract infection (U.R.T.I.), febrile catarrh, acute respiratory disease (A.R.D.) and coryza. Stuart Harris et al (1938), in a Medical Research Council report on epidemic influenza considered it necessary to clarify the position of influenza to distinguish it from certain other conditions that confused the situation by resembling epidemic influenza to some extent. These confusing symptoms were described as febrile catarrh. Another caution that was put forward by these workers was to avoid the use of mixed terms such as "Influenzal colds" in favour of simple terms such as coryza and pharyngitis.

This /

This indicates the confusion existing at that time in the field of upper respiratory diseases and no attempts were made to clarify the situation until the beginning of the second world war. During the war, however, the problem began to receive attention because of the necessity to hospitalize a vast number of recruits under training and soldiers in the field with upper respiratory complaints. A Commission was then set up by the United States government to look into the matter and it published its preliminary report on "Acute Respiratory Diseases among new army recruits" at Fort Bragg in 1946. The Commission observed that 90% of the illnesses were some form of acute respiratory disease (A.R.D.). In another study published by the Commission in 1947 work was conducted on the experimental transmission of minor respiratory illness to human volunteers, and the following clinical entities were considered.

1. Undifferentiated acute respiratory disease.
2. Severe common cold.
3. Common cold.
4. Bronchitis resembling atypical pneumonia.
5. Minor respiratory illness.

These clinical conditions were selected for the study because they could be considered as definite individual entities and the diagnosis was made on exclusion and not on the direct consideration of symptoms. (Commission 1946, 1947).

In /

In 1948 Dingle described three unaccounted-for respiratory symptoms.

1. Common cold.
2. Non bacterial exudative tonsillitis and pharyngitis.
3. Undifferentiated acute respiratory disease.

Dingle pointed out the now regularly accepted fact that more than one agent may produce the same clinical type of illness, and in addition a single agent, such as influenza A virus, may induce diverse clinical pictures simulating almost any of the common diseases of the respiratory tract. (Dingle, 1948).

Stuart-Harris (1953) considered that in terms of aetiological agents there were four chief clinical syndromes which together formed the group of virus infections of the respiratory tract in man.

1. Influenza due to various influenza viruses.
2. Common cold due to the cold viruses.
3. Atypical pneumonia due to known virus and rickettsial agents.
4. Acute undifferentiated respiratory disease, or febrile catarrh.

The last named included exudative pharyngitis, acute respiratory disease and primary atypical pneumonia of uncertain aetiology.

This was the era when from the vast number of viruses which were later on proved to be associated with respiratory symptoms only one group, the influenza viruses, was known. As a result of this gap between the occurrence /

occurrence of certain distinct symptoms and the nature of their exact aetiological agents the clinicians and virologists continued to use the same terminology and criteria for about another decade with little or no variation.

Dingle and his colleagues in 1953, in their studies in a group of Cleveland families, were the first to consider the problem in its present perspective by using only two terms; "Common respiratory disease" to include common cold, rhinitis, laryngitis, bronchitis, and other respiratory diseases of undifferentiated type and "Specific respiratory disease" to include streptococcal tonsillitis and pharyngitis, primary atypical pneumonia, pneumococcal pneumonia and influenza. In the second group influenza was the only disease of known virus aetiology. (Dingle et al., 1953).

In 1955 Hilleman and others in their study on the epidemiology of RI (RI-67) group respiratory virus infections, considered the following entities; acute pharyngitis, tonsillitis, bronchitis, pneumonitis or simply upper respiratory illness. Designations such as common cold, rhinitis or tracheitis were used less frequently. (Hilleman et al., 1955a). Grieble et al (1958) in their study of the aetiology of common respiratory infections considered that the differentiation into distinct clinical syndromes was valuable in its diagnostic and therapeutic /

therapeutic implications. As proof, they pointed out that the clinical identification of streptococcal pharyngitis was made in 60%, while those of specific viral agents in 20%. However persons suffering from one or other of the detectable viral infections could be distinguished as a group quite clearly from persons with acute afebrile coryza or undifferentiated upper respiratory infections of unknown aetiology.

VIRUSES ASSOCIATED WITH UPPER RESPIRATORY TRACT INFECTIONS.

Although the first viruses were discovered at the end of the last century there were no attempts at that time to isolate viruses from cases of upper respiratory disease. This was probably because many of the respiratory diseases that were differentiated as entities now known to be of viral aetiology had already been ascribed to one or another species of bacteria.

EARLY PROOF OF A VIABLE AGENT BY THE TRANSMISSION OF RESPIRATORY DISEASE TO VOLUNTEERS.

Kruse (1914) succeeded in producing coryza by intranasal inoculation of filtrates of the nasal secretions from acute coryza into 15 out of 30 subjects. This experiment was later successfully repeated by Foster (1917) who obtained positive results in 7 out of 10 inoculations.

Dold in 1917 succeeded in transmitting the common cold to Chinese students living under ordinary conditions but was unsuccessful with hospital patients. (Dold 1917).

The success of Kruse, Foster etc with coryza, led to similar attempts with influenza and Selter (1918) produced slight influenza-like symptoms after a one day incubation period in two subjects sprayed for half an hour with a filtrate of throat washings from a case of influenza. During the peak of the epidemic Leshke (1919) /

(1919) was able to produce typical influenza in "three men sprayed with cultures from filtered sputum, throat washings and lung juice of influenza cases". (M.R.C. 1929).

Olitsky and McCartney (1923) reported the transmission of a clinical condition similar to a typical common cold from man to man with filtered nasopharyngeal washings.

In 1930 Dochez, Shibley and Mills confirmed the experimental transmission of the common cold to man and anthropoid apes. (Dochez, Shibley and Mills, 1930).

Isolation of respiratory Viruses.

Shope (1931) reported the existence of a filterable virus associated with Haemophilus influenzae suis in swine influenza. Dochez et al. (1931) reported the cultivation of the common cold virus in chick embryo tissue culture.

In 1933 the first positive isolation of influenza virus was made in ferrets by Smith, Andrewes and Laidlaw from cases of influenza, and it was found that no such relationship as exists between swine virus and H. influenzae suis is necessary. (Smith, Andrewes and Laidlaw, 1933).

In 1934, Andrewes, Laidlaw and Smith reported the transmission of swine and human influenza viruses into mice and their neutralization with specific antisera. This was confirmed in the same year by ~~Smith~~ Francis and the virus is /

is now known as Type A. (Andrewes, Laidlaw and Smith, 1934, Francis 1934).

At this time neutralization tests with convalescent or immunized animal sera showed that swine influenza virus was antigenically related but not identical with, influenza A. virus from man (Francis and Shope, 1936). Later, antigenic differences were shown to occur between different strains of influenza A virus (Magill and Francis 1936; 1938, Francis and Magill, 1938, Smith and Andrewes, 1938). In 1940 a completely new antigenic type of influenza virus was isolated from the current epidemic and named influenza type B. (Francis 1940, Magill, 1940).

Another type of influenza virus isolated by Taylor, (1949) was identified by Francis et al. (1950) and called influenza virus C. In 1959 human influenza A viruses were further classified by the World Health Organization A (1933-46), A1 (1947-56) and A2 or Asian (1957). (W.H.O. Tech Rep. Series No 170, 1959).

At this stage it seems appropriate to give a brief outline of our present knowledge of upper respiratory virology, and then to follow the progress of various groups of viruses in detail to their present status in respiratory virology. 1950 stands out as the turning point, because after this year many new viruses were isolated, and by 1960 most of the viruses associated with the present problem had been isolated, identified and grouped. /

grouped.

VIRUSES ENCOUNTERED IN RESPIRATORY TRACT INFECTIONS.

Respiratory symptoms may be caused by both RNA and DNA viruses. Among the RNA viruses the myxoviruses and rhinoviruses are the important respiratory pathogens, while the enteroviruses and reoviruses have also been isolated from time to time from the respiratory tract as the cause of acute febrile or mild respiratory illness. (W.H.O. Tech Rep. Series 170, 1959).

Of the DNA viruses adenoviruses and herper simplex virus are the most important isolates associated with overt respiratory symptoms. Various other viruses such as variola, rubella, and varicella have been isolated from the respiratory tract without apparently being related to respiratory disease.

R.N.A. VIRUSES.

Myxoviruses:-

Myxovirus was the group name given to influenza A virus and its related viruses in 1955 by Andrewes and his co-workers, (Andrewes et al., 1955). Later on new viruses were included in the group which is now divided into two sub groups.

I. Influenza viruses, types A, B, C, (Type A divided into Human Porcine, Equine and Avian sub types).

II. Paramyxoviruses, including Newcastle disease, mumps, para-influenza, 1, 2, 3 and 4 and Simian myxoviruses SV5 and SV41.

(Andrewes /

(Andrewes and Pereira, 1967).

The myxovirus particles consist of a core of nucleoprotein surrounded by an outer membrane and are between 60 to 200 millimicrons in diameter. Uniformity in size is more consistent in subgroup one than in subgroup two.

Most members of the group adsorb to the surface of red cells of fowls and some other vertebrates causing their agglutination, due to the presence of enzymatic groups called haemagglutinins on the surface of the viruses.

More recently it was found out that some members of the group demonstrated the property of haemadsorption in which red cells from various animals and man adhere to the tissue culture infected with the viruses (Vogel and Shelokov 1957).

The properties of haemadsorption and haemagglutination are inhibited by certain mucoproteins present in mammalian sera and other biological fluids. In some viruses otherwise related to the myxoviruses haemadsorption and haemagglutination may be absent (rinderpest and respiratory syncytial viruses). In 1966 Waterson and Almeida suggested the name pseudomyxoviruses for the measles, distemper, rinderpest triad and some bovine parainfluenza 3 viruses. Andrewes and Pereira in 1967 have called their second subgroup unofficially the paramyxoviruses and have dealt with the measles, distemper and rinderpest triad and the respiratory syncytial virus under /

under a separate heading of "Other RNA Viruses". (Waterson and Almeida 1966, Andrewes and Pereira 1967).

Sub Group I:- Influenza A, B and C. viruses.

These are the viruses whose aetiological role has been established in clinical influenza. Except for an occasional overlapping during the epidemics they are not the basic concern of the present study. Their isolation and general properties have already been mentioned.

Sub Group II.

Andrewes et al (1959) suggested the name para-influenza viruses for the group of newly-described myxo-viruses; among these he included

(a) Sendai virus (Haemagglutinating virus of Japan, Influenza virus D). = para-influenza 1

(b) Croup associated virus (CA) virus (Acute laryngo-tracheo bronchitis virus). = para-influenza 2.

(c) Haemadsorption virus I. = para-influenza 3.

(d) Haemadsorption virus 2. = para-influenza 1.

The four viruses are closely related and tend to be somewhat larger than influenza A, B and C viruses. They haemolyse certain erythrocytes. The haemagglutinating property may not be readily demonstrable in fresh isolates until they have been adapted to laboratory growth conditions, however the virus is readily demonstrated by haemadsorption. In these respects they also resemble the mumps and Newcastle /

Newcastle disease viruses. They show common antigens and appear to comprise a group in which each member is related to at least one other member and frequently to two or more members of the group.

Later on another virus M-25 isolated by Johnson et al. in 1960 was included in the Group and is now called para-influenza 4 virus. (Johnson et al., 1960).

Para-influenza I. (Sendai, Haemadsorption virus 2, New born pneumonitis virus, Haemadsorption virus of Japan, Influenza D.)

In 1953 the Sendai virus was isolated from new born children with pneumonitis in Japan (Kuroya et al., 1953). The virus was later on shown to be prevalent in the U.S. (Jensen et al 1955, Francis 1955), and on the basis of similarity of characteristics of the virus it was tentatively designated as Influenza D virus (Francis 1955). Gardner (1957) demonstrated an antigenic relationship between the Sendai virus and Mumps virus and Chanock et al., (1958) showed that the newly isolated Haemadsorption virus (HA2) showed a common antigen with Sendai virus and could not be distinguished from it by Haemagglutination inhibition and neutralization techniques.

In 1957 Bruce White, Gardner and Hope Simpson from London and Sommerville from Glasgow reported the occurrence of antibodies to Sendai virus in Britain. (Bruce White et al 1957, Sommerville 1957). Andrewes et al. in 1959 proposed the inclusion of both Sendai and Haemadsorption 2 viruses in Para-influenza I. Zhdanov (1960) however had argued in favour of their separation. As the Sendai virus is well known to cause latent infection in laboratory mice (Chun et al 1956) some workers doubt the claims of its being the cause of pneumonitis in new born children etc. HA2 strains on the other hand have been isolated only from man, and have been shown to be definitely /

definitely associated with respiratory disease. (Chanock et al 1958, Vargosko et al 1959, Tyrrell et al 1959, Dick et al 1961).

Para-influenza 2:- Croup associated (CA) virus was isolated by Chanock in monkey kidney tissue cultures in Cincinnati and by Beale et al in Hela and human amnion cultures in Toronto from infants with croup during the winter of 1955, (Chanock 1956, Beale et al., 1958). Five of eleven infants studied in Cincinnati developed antibody to CA virus and the virus was isolated from ten out of fifteen infants studied in Toronto. Chanock (1956) showed that the strains isolated in the two places were indistinguishable.

Five viruses isolated in Washington D.C. from children during October 1955 were identified (Vogel et al., 1958) as CA virus.

The properties of the virus place it in the Myxovirus group and the syncytial formation on monkey kidney and human amnion tissue cultures is shared with the mumps virus to which it has minor antigenic relationships.

Para-influenza 3:- Haemadsorption virus I.

The virus was isolated from children with respiratory illness by Chanock et al in 1958 using the haemadsorption technique in monkey kidney tissue culture. The virus was recovered from 35 children, of whom 8 were hospitalized /

hospitalized with pharyngitis, bronchiolitis and pneumonia and 27 were involved in an outbreak of febrile respiratory disease in a nursery, (Chanock et al., 1958).

Para-influenza 4:- Was isolated from an adult college student with mild pharyngitis and coryza and 30 infants and small children with or without clinical respiratory illness (Johnson et al 1960) and initially named as M - 25.

Paired sera from these individuals revealed significant rise in levels of neutralzing antibody. The properties of the virus resemble other myxoviruses, complement fixation tests with guinea pig antisera indicate that the virus is antigenically distinct from influenza, mumps, Newcastle disease and para-influenza 1, 2 and 3 viruses. Studies suggest that there is a relationship between M-25 and mumps virus in that sera from adults but not children infected with mumps virus, showed increased amounts of complement fixation for M-25 virus. Presently two subtypes are recognised subtype A (M-25) and subtypes B (CH 19503), (Canchola et al 1964).

Many studies of respiratory infections have provided evidence of infection with the para-influenza viruses in Britain.

Bruce White et al., (1957) first reported an outbreak of respiratory illness from England with rising titres for Sendai virus. At the same time Gardner from England and Sommerville from Glasgow reported the existence of antibodies to the Sendai virus. (Gardner, 1957, Sommerville, 1957).

This was followed by Grist and Sommerville's (1959) report of the production of antibodies to Sendai virus /

virus in respiratory virus infections in the Glasgow area. In the same year Tyrrell et al (1959) reported the production of respiratory symptoms in volunteers inoculated intranasally with para-influenza 1 and 3 viruses. Gardner et al (1960) produced evidence of infection with Sendai virus and for the first time implicated the Croup associated virus (Para-influenza 2) with respiratory disease in this country. Holland et al (1960) demonstrated the presence of para-influenza virus 1 and para-influenza virus 3 in patients with respiratory disease.

Sutton (1962) reported the outbreak of respiratory illness due to para-influenza 3 virus in a Sheffield nursery, and in the same year Heath et al detected the presence of inhibitors of Sendai virus haemagglutination in the human population and also found significant antigenic relationships between the titres against Sendai virus and those against HA1, (para-influenza 3), HA2, (para-influenza 1), and C.A. (para-influenza 2) and mumps virus.

Tobin (1963) reported the isolation of para-influenza 1 virus from hospitalized children and the isolation of para-influenza 1 and para-influenza 3 was reported from a hospital patient in Manchester, in 1964, (Tobin 1963), (P.H.L.report Manchester 1964).

Clarke et al., (1964) reported the isolation of para-influenza viruses 1, 2 and 3 from upper respiratory tract /

tract infection, laryngotracheitis, bronchitis and broncho-pneumonia from hospital and general practice patients.

There are many reports of infection with para-influenza viruses in general practice (Higgins et al 1963, 1964, Banatvala et al 1964) and Banatvala et al (1965) reported the isolation of para-influenza viruses from 18% of all acute respiratory infections in Cambridge general practice.

The Medical Research council of Great Britain has published its report on "a collaborative study of the aetiology of acute respiratory infection in Britain", (M.R.C. 1965) which mentions the isolation of para-influenza viruses from children and adults the viruses include three types only 1, 2, 3.

Para-influenza 3 has been reported to be the cause of an outbreak of respiratory illness in a children's home. (Aitken et al., 1967).

Higgins (1966b) summed up the results of their studies from 1961 to 1965 and mentioned the isolation of para-influenza types 1, 2 and 3 only.

So far para-influenza virus 4 has not been reported in Britain.

Respiratory syncytial virus (R S Virus) :- Probable myxovirus.

Except for its inability to haemagglutinate or haemadsorb the other characters suggest that this is a myxovirus.

The virus was first isolated by Morris, Blount and Savage in 1956 from a chimpanzee suffering from coryza, (Morris et al 1956). Later on Chanock et al (1957) isolated it from infants with bronchopneumonia and in 1957 Chanock and Finburg suggested the name respiratory syncytial virus (RS), (Chanock and Finburg 1957). Huebner et al (1958) listed it as a cause of Croup and pneumonia in children. Beem et al (1960) impressed the need of avoiding freezing and thawing of the material from which this virus is to be isolated.

Peacock and Clarke first reported the isolation of this virus in Britain (Peacock and Clarke 1961). Since then the virus has also been reported from Australia, (Lewis et al 1961), Denmark, (Hornsleth and Volkert 1964) and a syncytial agent from France (Chany et al 1958).

In 1961 (Hamparian et al 1961a) reported the clinical illnesses associated with the virus as, acute respiratory illness, bronchitis, bronchiolitis, bronchopneumonia and early Croup. Chanock et al (1962) found it to be responsible for a considerable proportion of the severe respiratory illnesses that afflict infants and /

and children. Reinfection can occur later on in life and is probably associated in a proportion of instances with a mild respiratory illness.

At the present stage of our knowledge we can say as a result of many studies carried out in this country and elsewhere that respiratory syncytial virus is the single most important pathogen of infants and young children who are affected by it with lower respiratory symptoms. In older children and adults it causes minor upper respiratory symptoms and acute exacerbations of chronic bronchitis.

(Hilleman et al 1962, Sommerville 1963, Higgins et al 1963, Tobin 1963, Hambling 1964. P.H.L.S. Manchester 1964, Gardner et al 1964, Wulff et al 1964a, Holzel et al 1965, Urquhart et al 1965, Medical Research Council Report 1965, Chanock and Parrott 1965, Higgins et al 1966 and Aitken et al 1967).

The virus is inactivated swiftly at 20°C (Wulff et al 1964b) but can be stored satisfactorily with the addition of 50% glycerine. Perhaps this inability to survive the transport and isolation procedures generally used for other viruses accounts for the low isolation rates of many studies.

Rhinoviruses.

Attempts to isolate a virus from the common cold date back to the early work of Kruse in Germany in 1914. Foster (1917) confirmed Kruses work and also claimed to have cultivated an agent anaerobically. Work was carried out extensively by Dochez and colleagues in the thirties who infected human beings and chimpanzees with common cold filterates. (Dochez et al 1931, Dochez et al 1936a Dochez et al 1936b). Hopeful results were obtained by Andrewes et al (1953) when they successfully passed a virus serially for ten subcultures in cultures of human embryonic lung tissue and produced colds in human volunteers.

Actual success in cultivation was only achieved when the right cultural conditions for growing the virus were discovered (Tyrrell et al 1960, Hitchcock and Tyrrell 1960, Tyrrell and Parsons 1960 and Tyrrell and Bynoe 1961). The requirements proved to be the use of human embryonic kidney cells in a medium of low bicarbonate content pH7.0, and incubation at 33°C on a roller drum. Under these conditions several viruses designated as rhinoviruses were cultured from common colds and tested in human volunteers (Bynoe et al 1961). Most strains now designated as H strains grow only in human embryo kidney or human embryo lung cells, but a few the M strains, can grow in monkey kidney cells and also in the cancer cells lines Hela /

Hela and HEp. 2. Some strains can also be isolated in the cultures of diploid cells of the human embryo lung (Hayflick and Moorhead 1961), (Tyrrell et al 1962). Tyrrell and Bynoe (1966) introduced an organ culture technique using fragments of ciliated epithelium capable of isolating new H viruses and Higgins (1966a) has utilised it very successfully.

The H and M strains differ antigenically from each other as determined by the cross-neutralization tests, and within each group there is a large measure of heterogenicity, (Taylor Robinson and Tyrrell 1962 a and b). This was confirmed by Hamre and Procknow and Hamparian, Ketler and Hilleman in 1961 in the U.S.A. when they described their discovery of the agent then known as Coryza virus. (Hamre and Procknow 1961, Hamparian Ketler and Hilleman 1961).

The rhinoviruses are 20 to 30 millimicrons in size and have a core of RNA. They are ether resistant and can survive at 4°C for four weeks, but are inactivated quickly at pH5.3. This property lends to acid lability which is their distinguishing character from the entero-viruses. (Tyrrell and Chanock 1963, Andrewes and Pereira 1967). There are at least 80 strains of H rhinoviruses and the first 55 which have been fully compared have been numbered by Kapikian et al (1967). One virus originally named JH and 2060 and finally typed as ECHO 28 antigenically /

antigenically overlaps with one M strain B632 and is now included in the rhinovirus group. (Price 1956, Pelon et al 1957, Price et al 1959, Jackson et al 1960, Kendall et al 1962, Andrewes and Pereira 1967).

Volunteer Studies in Britain:-

Studies have been carried out using a large number of human volunteers at the Common Cold Research Unit, Salisbury since its establishment in 1946.

The work carried out with human volunteers initially included the transmission of "wild" colds.

After the first limited success of 1953 human volunteers have been needed to prove the laboratory isolations and the study of the behaviour of virus from common colds.

Comparative accounts of the work at the Common Cold Unit have been published from time to time, (Andrewes 1949, 1950, Andrewes et al 1951, Andrewes et al 1953 and Andrewes 1958, 1962, 1966a and 1966b).

Other studies needing human volunteers in this country and more often than not connected with the Common Cold Unit include the confirmation of virus studies (Tyrrell et al in 1960, Tyrrell and Parsons 1960, Tyrrell and Bynoe 1961, Taylor Robinson and Tyrrell 1962a, Tyrrell and Bynoe 1965), and the serological studies on sera from Merseyside volunteers, (Taylor Robinson and Tyrrell 1962b).

Enteroviruses.

The similarities of enteroviruses to rhinoviruses and their steady association with respiratory symptoms has made it essential to include them for consideration in this study.

Various serotypes of Coxsackie A viruses have been aetiologically implicated in upper respiratory disease. Huebner et al (1952) drew the attention towards a possible role for both Coxsackie A and B viruses in upper respiratory symptoms. Coxsackie A viruses have constantly been responsible for herpangina commonly manifested by febrile pharyngitis, (Parrott 1957, Lennette et al 1958, Paffenbarger et al 1959, Kendall et al 1960, Huebner 1963, Kibrick 1964). The same herpangina - associated Coxsackie A viruses have been associated with mild upper respiratory disease (Huebner 1963). Coxsackie A10, besides causing herpangina, has been associated with lymphonodular pharyngitis in which sore throat is a common complaint, (Steigman et al 1962). Strains of Coxsackie A21 (Coe) virus have regularly been isolated from the pharynx during mild undifferentiated respiratory illness, and the presence of neutralizing antibody has been shown to confer protection, (Chanock and Johnson 1961, Johnson et al 1962).

Undifferentiated febrile illness with cough and pharyngitis has been reported during attacks of Coxsackie B /

B virus infections (Paffenbarger et al 1959).

Coxsackie B2 isolations were significantly higher in persons with mild febrile respiratory illness than in unaffected subjects. (Heggie et al 1960). Similar illness has been reported due to Coxsackie B3, (Kendall et al., 1960, Bell et al., 1961), Coxsackie B5 (Vargosko et al., 1962).

Infection with Echo viruses has frequently been accompanied by respiratory symptoms (Kibrick, 1959). Acute febrile respiratory disorders were the most common symptoms associated with Coxsackie and Echo viruses during a longitudinal study in an orphanage (Kibrick, 1959), Echo 20 (JV-1) virus was isolated from illness primarily characterized by mild respiratory signs (Cramblett et al., 1958, Rosen et al., 1958). Volunteer inoculation of Echo 20 in Salisbury produced headache, malaise, aching limbs, sore throat and fever as well as common cold symptoms. (Buckland et al., 1961). Besides the serotypes mentioned above respiratory symptoms have been associated with Echo 6, Echo 8, Echo 11 and Echo 28, which has now been transferred to the rhino virus group.

Enteroviruses encountered in respiratory infections have been reported from Britain since 1959.

Pereira and Pereira in 1959 isolated the Coe virus (Coxsackie A21) in Britain from persons with respiratory symptoms and in the same year Buckland et al performed volunteer /

volunteer studies with Echo type 11 virus. (Pereira and Pereira 1959, Buckland et al 1959).

Kendall and his colleagues (1960) reported the isolation of Coxsackie B_3 and Coxsackie B_4 viruses from respiratory infections in children and Holland and others (1960) reported the isolation of Coxsackie B_3, Coxsackie B_5 and Echo type 3 virus from children with respiratory disease.

Buckland et al (1961) carried out volunteer study with Echo type 20, and Kendall et al (1962) reported the isolation of Echo type 28 and B632 two antigenically overlapping viruses, one belonging to the Echo and the other to the rhinovirus group.

McDonald et al (1962) reported the presence of Coe (Coxsackie A21) virus in a R.A.F. camp, and in the same year Heath and co-workers found titres against Echo type 11 in volunteers. (Heath et al 1962).

In 1962 Higgins et al reported the isolation of polio virus and Coxsackie B3 viruses in their studies of acute respiratory disease in a general practice. (Higgins et al 1963).

In 1964 isolation of enteroviruses was reported from upper respiratory tract infections, tonsillitis, croup and lower respiratory tract infections from the Public Health Laboratory in Manchester. (P.H.L.S. report Manchester /

Manchester 1964) and Clarke et al (1964) reported the isolation of Coxsackie A2, Echo type 12 and poliomyelitis type I viruses in patients with acute respiratory infection. Holzel et al (1965) reported the isolation of picorna viruses from respiratory cases. Urquhart et al (1965) reported the isolation of Echo types 11, 8, 12, 14 and 15 from respiratory infections in children in Edinburgh.

Higgins et al (1966) mentioned poliomyelitis Virus I and II, Coxsackie B viruses types 1, 2, 3, 4, 5, 6 and Coxsackie A virus types, 2, 4, 5, 6, 9 and 10 being isolated from respiratory patients in a general practice.

Reoviruses.

The first virus that subsequently was classified in the reovirus group was isolated by Stanley et al in 1953, who called it Hepato-encephalomyelitis virus (HEV). (Stanley et al 1953). The name "Reovirus" was first suggested by Sabin (1959) to include Echo 10 and antigenically related viruses. The term "REOVIRUS" is obtained from the initial letters of Respiratory Enteric Orphan viruses. They differ from all other enteroviruses in their larger size, being 60 - 90 millimicrons in diameter, and different cytopathic effect, the formation of an RNA containing cytoplasmic inclusion being characteristic (Tournier and Plissier 1960, Malherbe and Harvin 1957, Shaver, Barron and Karzon 1958). Most of the workers agree on there being 92 Capsomees (Mayer et al 1965, Loh et al 1965). Van Tongern (1957) in Holland isolated a similar agent. Rosen (1960) reported that the simian viruses known as SV 12 and SV 59 were identical with reovirus types 1 and 2 respectively. Stanley (1961a) has shown that HEV virus was identical with Echo type 10. Three serological types have been designated by using the haemagglutination inhibition technique Rosen (1960) and Hartley, Rowe and Austin (1962) have divided 8 strains of type 2 into 4 subtypes.

Human infection has been shown to occur with each of the three types. (Stanley et al 1954, Stanley 1961b, Stanley /

Stanley and Leak 1963, Stanley et al 1964). Clinical illness supported by viral isolation has been described in children only (Stanley 1961b, 1964). The outbreaks described by Stanley and others have been in nurseries and creches and the illness has usually been mild and febrile involving the gastrointestinal and upper respiratory tract. (Rosen et al 1960 Stanley 1964).

The association between reoviruses and the following conditions is much less certain; pneumonitis and alopecia in an infant (Stanley et al 1953), a fatal case of disseminated encephalomyelitis (Krainer and Arnson 1959), and a common cold syndrome (Jackson et al 1961, 1963).

So far the reoviruses have not been isolated from respiratory infections in Britain, but antibodies to reovirus type 1 and type 2 have been encountered in British adults by Taylor Robinson (1965).

DNA Viruses.

Adenoviruses:- The first isolation of an adenovirus was reported by Rowe et al (1953) from human adenoids undergoing spontaneous degeneration in tissue cultures, and was called the "adenoid degeneration-agent". Hilleman and Werner (1954) isolated a new agent from patients with acute respiratory disease. The agent showed immunological relationship with Rowes adenoid degeneration agent and was named R167.

Huebner et al (1954) showed that at least six immunologically distinct types existed. These were all related by a common group specific complement fixing antigen. The authors suggested Human Adenoidal-pharyngeal conjunctival (APC) virus as the name of the group. RI-67 was demonstrated to be a member of this group and was assigned the designation of type 4. Another agent isolated by Neva and Enders (1954) was shown to belong to type 3 of the group. Parrott et al (1954) described a hospital outbreak of pharyngitis with conjunctivitis and suggested that the observed syndrome was a new specific clinical entity related aetiologically to APC virus type 3. This clinical entity was later on renamed by Bell et al, (1955) pharyngo-conjunctival fever. In 1956 the term "Adenovirus" was agreed upon by Enders et al to replace the various names given to the viruses by different workers. Since then a vast amount of literature /

literature has accumulated about the Adenoviruses showing their epidemiologic and clinical associations. (Enders et al 1956).

Adenovirus type 3 has been found to be the aetiological agent of non-bacterial pharyngitis with or without conjunctivitis. (Parrott et al 1954, Bell et al 1955, Ginsberg et al 1955, Cockburn et al 1956, Jawetz et al 1956 , Grayston 1957, Jordan 1957, Loosli 1957, Rowe et al 1957, Jawetz 1957, Kendall et al 1957, Huebner 1957, Hilleman 1957, Van Horne et al 1957, Jordan et al 1958, Bell et al 1962, Pereira et al 1963).

Adenovirus type 4, 7, 14 and 21 are often responsible for acute respiratory disease (ARD) and patients with this syndrome are often reported to complain of sore throats. (Hilleman et al 1955a, Berge et al 1955, Descombe and Hilleman 1956, Rowe et al 1956, Woolridge et al 1956, Pereira et al 1963).

Types 1 and 5 have been isolated from acute pharyngitis in adults and children. (Huebner et al 1954, Woolridge et al 1956, Grayston 1957, Loosli 1957, Rowe et al 1957, Werner et al 1957, Jordan et al 1958, Merchant et al 1958, Parrott 1963).

Type 2 has often been isolated from upper respiratory symptoms in children, sometimes with influenzal and conjunctival symptoms. (Rowe et al 1957, Werner et al 1957, Jawetz 1957, Jordan et al 1958, Parrott 1963).

Type /

Type 8 has been isolated from epidemic kerato-conjunctivitis with occasional isolation of type 6 and 10. (Jawetz et al 1955, Loosli 1957, Rowe Huebner and Bell 1957).

Types 9, 11, and 12 have been isolated from stools in poliomyelitis surveys (Loosli 1957). Types 26 and 27 have been shown to cause conjunctivitis in volunteers in the absence of antibodies. (Knight et al 1963). The role of these and many others has yet to be demonstrated in human or animal diseases.

Total 31 serotypes have been mentioned in the literature (Swain and Dodds 1967) and besides the human types there are bovine, porcine and Avian types.

Adenoviruses were first isolated in Britain in 1955 (Zaiman et al 1955), from adenoids removed in Sheffield.

Andrews et al (1956) reported the occurrence of APC virus as the group was then called in an R.A.F. station in England. Kendall et al (1957) reported a school outbreak of pharyno-conjunctival fever associated with adenovirus types 3, 7 and 14. Stovin et al (1958) reported sporadic acute respiratory infections in adults due to adenovirus types 1 - 7, 9 and 10. Holland and colleagues (1960), reported the isolation of adenovirus types 1, 2, 3 and 5 from children in a general hospital. Kendall et al (1960) also isolated adenovirus types 1, 2, 3, 4 from children in general practice. In the same year

(Gardner /

(Gardner et al 1960) reported the isolation of adenovirus from Newcastle in association with upper respiratory infections and pneumonia.

Sutton (1962) reported the isolation of adenovirus types 1, 2 and 5 from a residential nursery in Sheffield, but suggested these were nonpathogenic.

McDonald et al, (1962) reported that 26% of the admissions in their study of respiratory virus infections in R.A.F. were due to adenoviruses.

Higgins et al, (1963) reported the isolation of adenovirus types 1, 2, 3, 5, 7, 14 and 21 during their study of respiratory infection in general practice, during 1962 and again the isolation of types 1, 2, 5 and 21 in 1963. (Higgins et al 1964).

Tobin (1963) reported the isolation of adenoviruses in Manchester and Potter and Shedden (1963) reported the occurrence of adenovirus antibodies in normal children.

Clarke et al (1964) reported the isolation of adenovirus types 1, 2, 7, 21 from patients in a general practice.

Holzel et al (1965) reported the isolation of adenovirus from children with respiratory symptoms and in (1965), Urquhart et al reported the isolation of adenovirus types 1, 2, 5 endemic types and 3, 4, 7 and 14 epidemic types from City Hospital, Edinburgh.

The /

The 1965 Medical Research Council report, listed the isolation of adenovirus types 1, 2, 3, 4, 5 and 21 in their collaborative study, (M.R.C. Rep 1965).

Aitken and colleagues reported the isolation of adenovirus types 1, 2, 3 and 5 in an Edinburgh nursery, (Aitken et al 1967).

The above references represent a brief survey of the experience of British workers with adenoviruses. Many questions regarding these viruses still remain to be answered and with the ease and frequency that the adenoviruses are now being isolated in this country, and in fact the world over the time may not be far enough when it will be possible to talk more definitely about their role in upper respiratory infections.

Herpes Simplex Virus.

Herpes simplex virus with its numerous primary and recurrent manifestations inside the mouth, quite often involving the lymphatic glands, nose, tonsils etc, (Ruchman and Dodd 1950, Trice and Shafer 1953) and causing syndromes with names like, stomatitis (recurrent), acute lymphatic gingivostomatitis also known as catarrhal stomatitis (primary), acute lymphatic rhinitis (primary) many of which may clinically touch the borders of upper respiratory infections deserved a place in the study.

The virus has been constantly recovered from patients with respiratory symptoms. Jawetz et al (1955) reported it from a case of acute keratoconjunctivitis and supported the evidence of isolation with an increase in the antibody titre. Hamre et al, (1961) reported its isolation from the specimens from the student health service and hospitalized patients in their study of viruses implicated in acute respiratory disease in Chicago, from October 1958 to June 1959. Jordan (1962) in his paper on the ecology of respiratory viruses mentioned it in the group of respiratory viruses associated with major manifestations in other systems. Hale et al (1963) isolated the virus from an epidemic of herpetic stomatitis in nursery children and the signs included cervical lymphadenopathy and pharyngitis. (Higgins et al 1963, 1964 and /

and 1966) reported isolation of herpes simplex virus from respiratory infections in general practice. Clarke et al, (1964) reported the isolation from bronchitis, bronchiolitis and pneumonia. Urquhart et al., (1965) reported the isolation of this virus from the cases of aphthous stomatitis, bilateral conjunctivitis, mild upper respiratory infections, pneumonia and bronchitis. The M.R.C. working party isolated this virus from common colds, feverish colds, influenza, sore throat and croup and Aitken et al (1967) isolated this virus from nursery children with respiratory infections. Glazen et al (1967) isolated it in 11.1% of the cases of acute pharyngitis.

Another important factor which is not to be overlooked in the evaluation of the role of this virus in upper respiratory illness is the fact that after any primary infection local reactions may occur at intervals. These local reactions may depend on a variety of stimuli including fever, lobar pneumonia other respiratory infections, trauma etc. (Wilson and Miles 1964).

Reasons for the present investigation:-

From a review of the literature on the association between viruses and upper respiratory tract infections it is apparent that much work has been done in this country and elsewhere. The report published by the M.R.C. working party in 1965 gave the findings of a practically nation-wide survey involving many British workers, but questions still remain unanswered and it is for this reason that the present study of the aetiological and epidemiological factors associated with the sore throat syndrome has been planned.

The subjects of the study have been children in general practice and an attempt has been made to correlate the pattern of symptoms with the isolation of viruses. Serological studies were limited but proved particularly useful in evaluating an infrequently used method of obtaining blood samples. This method of obtaining blood for serological tests on paper dated back to Stapp and Bercks (1948) and had been used in plant pathology and veterinary serology till 1957. Kalter (1957) used it for poliomyelitis neutralization tests. Since then it had been used in poliomyelitis antibody surveys Green and Opton (1960) for fluorescent antibody technique (Anderson et al 1961) and in complement fixation and Haemagglutination inhibition tests for adenoviruses and measles virus (Brody et al 1964). It was hoped that this might prove of particular use in general practice.

PART II.

CLINICAL AND LABORATORY METHODS.

INITIAL EXPERIMENTS TO STANDARDISE TECHNIQUES.

Selection of swabs and transport medium

There have been reports about the anti viral activity of certain commercial swabs (Mair 1965). In view of this a series of simple experiments were designed to solve the problems of efficient transportation and storage of the specimens.

It is a difficult and arbitrary task to determine the concentration of virus obtainable from a diseased throat. A hypothetical concentration of virus in the throat was taken as I : 300 of an ordinary tissue culture fluid suspension of the virus. This was based upon the observation that in our laboratory under the proposed conditions of study only the following concentrations of virus had shown any recognisable CPE or haemadsorption up to the fifth day of inoculation:

Influenza virus A	10-3
Adenovirus I	10-3
Adenovirus 3	10-3
Herpes simplex virus	10-4

To study the effect upon these viruses the conditions had to be set so as to meet the requirements of all these viruses and yet provide a practicable way of transporting the specimens from the surgery to the laboratory and then storage up to the final inoculation into /

into tissue culture.

Three types of swabs were chosen for comparison.

1. Johnson's Gamma irradiated sterile swabs.
2. Exogen sterile disposable swabs.
3. Surgical swabs (prepared in the Wellcome Laboratory City Hospital).

It was calculated that on an average a swab soaked 0.2 ml of medium. Accordingly the viruses were diluted to give the following concentrations Neat, -1, -2, -3. The swabs were dipped into these virus dilutions and then transferred into 4.8 ml of transport medium in a bijou giving the following final concentrations.

Neat	1 : 25
-1	1 :250
-2	1 :2500
-3	1 :25000

Virus survival under various conditions in contact with different swabs and two transport media was to be observed.

Transport media.

1. Bovine serum albumen 1 per cent
   Medium 199
   Sodium bicarbonate 5% of 4.4 per cent
2. Calf serum 2 per cent
   Medium 199
   Sodium bicarbonate 5% of 4.4 per cent

Antibiotics /

Antibiotics were added to both media in the following concentrations before use: penicillin 250 units streptomycin 250 µgs and mycostatin 50 units per ml.

Conditions of transport:-

Although 4°C for 6 hours and then storage at - 70°C for 24 hours or more were the optimum practical conditions for this study a set of alternate conditions were also examined so that they may be used if this treatment proved to be impossible for the purpose of virus isolation.

1. Room temperature for 6 hours.
2. 4°C for 6 hours.
3. 4°C for 24 hours.
4. Room temperature for 24 hours.
5. 4°C for 6 hours followed by storage at -70°C for 24 hours or more.

Influenza A (RI5) in 2 per cent calf serum (Haemadsorption.) (Monkey Kidney Cells)

	Type of swab		
Treatment	Johnson's	Exogen	Surgical
R.T. 6 hours	4	6	5
4°C 6 hours	4	5	6
4°C 24 hours	6	5	5
R.T. 24 hours	2	4	2
4°C 6 Hrs -70° 24 Hrs	2	6	2

Influenza /

Influenza A (RI5) virus in 1 per cent Bovine Serum albumen (Haemadsorption) (Monkey Kidney Cells)

	Type of swab		
Treatment	Johnson's	Exogen	Surgical
R.T. 6 hours	4	4	4
4°C 6 hours	4	5	5
4°C 24 hours	6	6	4
R.T. 24 hours	4	4	2
4°C 6 Hrs -70° 24 Hrs	5	6	1

Adenovirus I in 2 per cent calf serum (CPE IN HEp 2)

	Type of swab		
Treatment	Johnson's	Exogen	Surgical
R.T. 6 hours	1	0	0
4°C 6 hours	0	0	0
4°C 24 hours	2	2	0
R.T. 24 hours	0	0	0
4°C 6 Hrs -70° 24 Hrs	2	4	4

Adenovirus I in 1 per cent Bovine serum albumen (CPE in HEp 2)

	Type of swab		
Treatment	Johnson's	Exogen	Surgical
R.T. 6 hours	2	1	0
4°C 6 hours	0	2	0
4°C 24 hours	0	4	2
R.T. 24 hours	0	0	0
4°C 6 Hrs -70° 24 Hrs	2	6	2

Adenovirus /

Adenovirus 3 in 2 per cent calf serum (CPE in HEp 2)

	Type of swab		
Treatment	Johnson's	Exogen	Surgical
R.T. 6 hours	2	0	0
4°C 6 hours	2	1	2
4°C 24 hours	0	0	0
R.T. 24 hours	0	0	0
4°C 6 Hrs -70° 24 Hrs	2	2	0

Adenovirus 3 in 1 per cent Bovine serum albumen (CPE in HEp 2)

	Type of swab		
Treatment	Johnson's	Exogen	Surgical
R.T. 6 hours	0	0	1
4°C 6 hours	0	1	2
4°C 24 hours	1	2	0
R.T. 24 hours	2	0	2
4°C 6 Hrs -70° 24 Hrs	0	4	0

Herpes Simplex virus in 2 per cent calf serum (CPE in HEp 2)

	Type of swab		
Treatment	Johnson's	Exogen	Surgical
R.T. 6 hours	0	0	0
4°C 6 hours	2	2	2
4°C 24 hours	0	1	0
R.T. 24 hours	0	2	1
4°C 6 Hrs -70° 24 Hrs	0	4	4

Herpes /

Herpes Simplex virus in 1 per cent Bovine serum albumen (CPE in HEp 2)

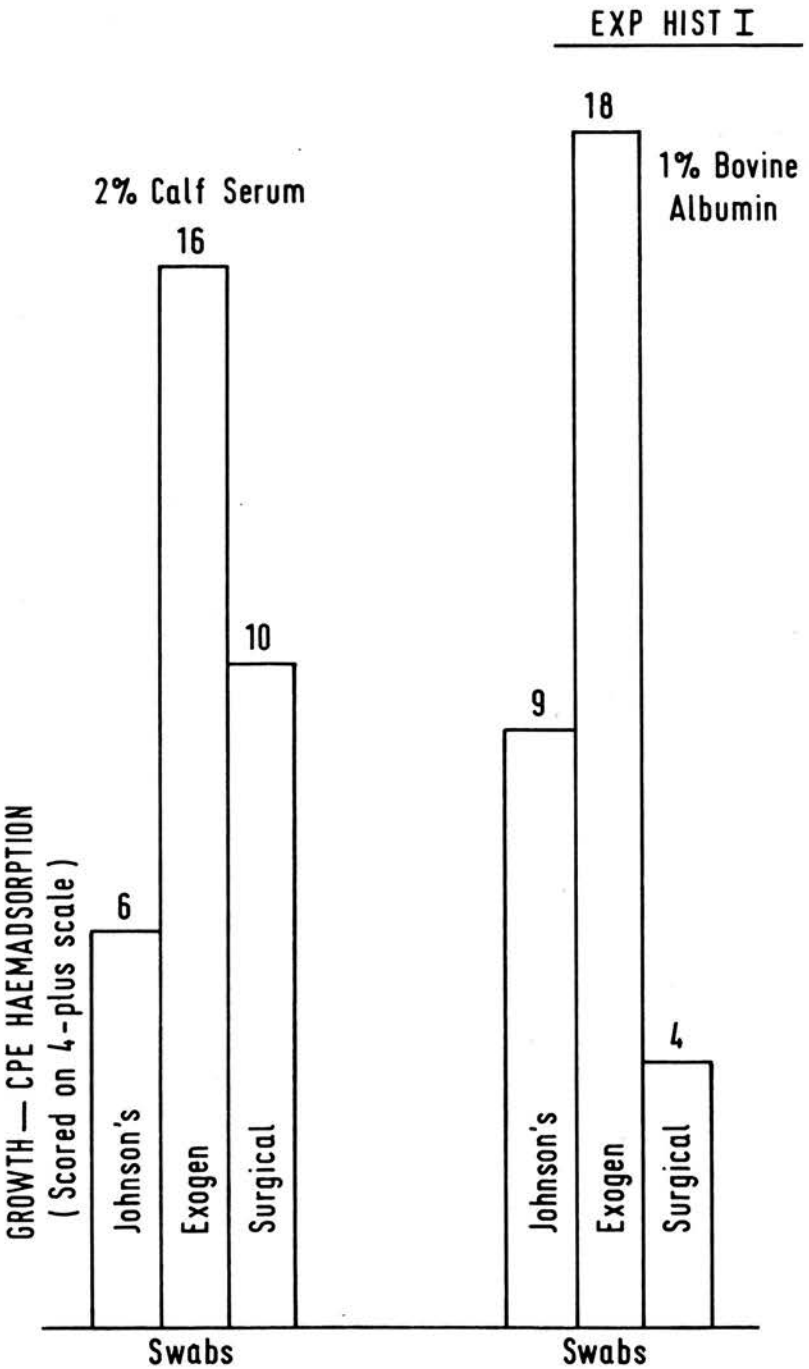
	Type of swab		
Treatment	Johnson's	Exogen	Surgical
R.T. 6 hours	4	3	3
4°C 6 hours	2	6	6
4°C 24 hours	2	3	2
R.T. 24 hours	0	0	0
4°C 6 Hrs -70° 24 Hrs	2	2	1

Results:-

The results were calculated from the number of pluses each virus scored in the two transport media on a four plus scale with its cytopathic effect or haemagglutination. The results as represented in (Experimental Histogram I) are the total number of pluses obtained by different viruses in combination with each swab and transport medium at the optimum conditions of transport and storage in appropriate cell systems.

Conclusions:

On the basis of these experiments Exogen swabs were used throughout. Transport medium containing 1% bovine serum albumen was used and the specimens were stored at -70° on arrival at the laboratory.



Growth of the test viruses with different types of swabs after storage in the two transport media at 4°C for 6hr followed by–70°C for 24 hrs or more.

EXPERIMENTAL HISTOGRAM I

Investigation of the Antiviral activity of Calf serum and Bovine serum albumen.

There are reports that the calf serum generally used in tissue culture media possesses some antiviral activity towards the myxoviruses. Bovine serum albumen was also used in the present study as an ingredient of the transport medium. Any adverse activity on the part of these two substances was therefore worth an investigation.

Three viruses, para-influenza 1, adenovirus 3 and herpes simplex virus were selected for the study.

Two sets of experiments were designed. In one all three viruses were diluted from $10^{-0.5}$ - $10^{-6.0}$ in either 1 per cent bovine serum albumen, 2 percent calf serum or Dulbecco A solution.

In the second experiment only para-influenza 1 virus was assayed in Monkey Kidney cells maintained on either calf serum +199 (as used in the study) or on Eagle's medium without calf serum. The dilutions were made in Dulbecco A solution.

Results:- These are shown in Experimental histogram II.

Para-influenza 1 virus:- In monkey kidney cells maintained in 199 + Calf serum Haemadsorption test with 0.4% human "O" red cells on the 4th day. Bovine serum albumen:- In bovine serum albumen diluted sample there was 4 plus haemadsorption up to 10^{-3} dilution, 3 plus haemadsorption up to $10^{-4.5}$ dilution 2 plus haemadsorption up to $10^{-5.5}$ dilution.

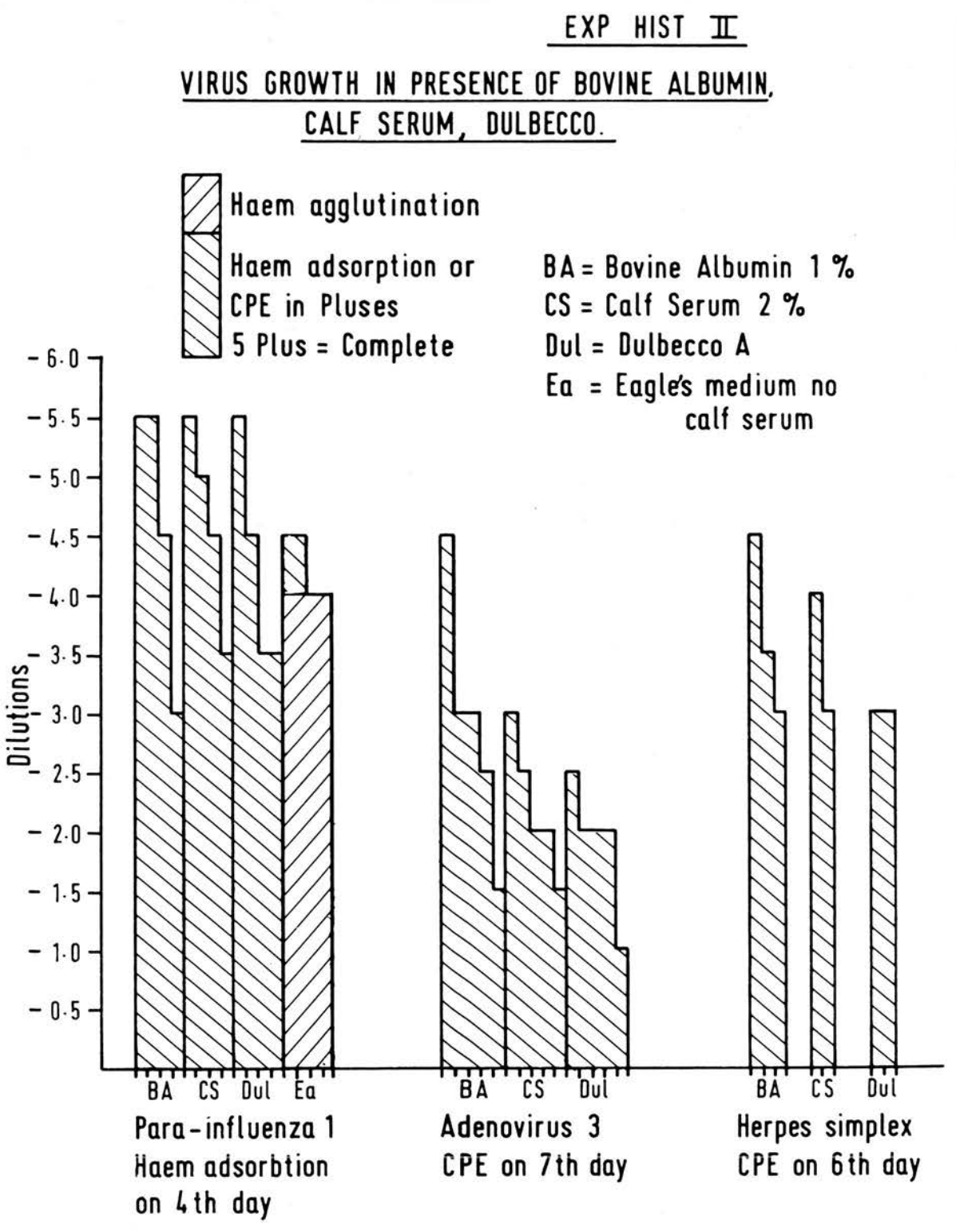
Calf serum:- In the calf serum diluted sample there was 4 plus haemadsorption up to $10^{-3.5}$ dilution. 3 plus haemadsorption up to $10^{-4.5}$ dilution, 2 plus haemadsorption up to 10^{-5} dilution and 1 plus haemadsorption up to $10^{-5.5}$ dilution.

Dulbecco A:- In dulbecco diluted sample there was 4 plus haemadsorption up to $10^{-3.5}$ dilution, 2 plus haemadsorption up to $10^{-4.5}$ dilution and 1 plus haemadsorption up to $10^{-5.5}$ dilution. Para-influenza 1 virus:- In monkey kidney cells maintained in Eagle's medium without calf serum. There was haemagglutination up to $10^{-4.0}$ dilution and 2 plus haemadsorption in $10^{-4.5}$ dilution.

Adenovirus:- In HEP 2 cells in 199 plus calf serum.

Bovine albumen:- In bovine albumen diluted sample there was complete CPE on the 7th day up to $10^{-1.5}$ dilution. 4 plus up to $10^{-2.5}$ dilution, 3 plus CPE up to $10^{-3.0}$ dilution and 1 plus CPE up to $10^{-4.5}$ dilution.

Calf Serum:- In calf serum diluted sample on the 7th day/



EXPERIMENTAL HISTOGRAM II

day there was a complete CPE up to $10^{-1.5}$ dilution, 4 plus CPE up to a dilution of 10^{-2} and a 2 plus CPE in the dilution of $10^{-2.5}$ and a 1 plus CPE in the dilution of 10^{-3}.

Dulbecco:- In the dulbecco diluted sample on the 7th day there was a complete CPE in the dilution of 10^{-1}, 4 plus CPE up to the dilution of 10^{-2} and a 1 plus CPE in the dilution of $10^{-2.5}$.

Herpes simplex virus:- In HEP2 cells in 199 plus calf serum.

Bovine albumen:- In the bovine serum albumen diluted sample on the 6th day there was a 3 plus CPE up to a dilution of 10^{-3}, a 2 plus CPE in the dilution of $10^{-3.5}$ and a 1 plus CPE up to the dilution of $10^{-4.5}$.

Calf serum:- In the calf serum diluted sample on the 6th day there was a 2 plus CPE up to the dilution of 10^{-3} and a 1 plus CPE up to the dilution of 10^{-4}.

Dulbecco A:- In the dulbecco diluted sample there was a 2 plus CPE up to 10^{-3}.

Conclusions:-

On the examination of results it is observed that when the viruses have come in contact with the three materials used bovine serum albumen has given the best chances of survival to the three viruses in general. Calf serum though not as good as bovine serum albumen is definitely better than plain Dulbecco A. As regards the specific effect /

effect of calf serum on the Myxovirus it appeared that the presence of calf serum in the medium has no effect on the haemadsorption activity of the cells in which the virus was being produced and thus gave a false sense of security. The production of haemagglutination in high degrees by the same virus in the absence of calf serum suggested the presence of better and more complete virus than in cell cultures propagated in media supplemented with calf serum.

Population under survey

Index cases:- Children between infancy and 15 years of age who attended the same medical practice have been included in this study.

Any child with a sore throat or with one or more of the clinical findings mentioned in form 1 (pain on swallowing, tonsillar exudate, cervical adenopathy, nasal discharge, pyrexia, vomiting, diarrhoea, conjunctivitis) in which a diagnosis of pharyngitis, follicular tonsillitis or of another specified nature had been reached was considered an index case.

The children were considered new index cases every time they attended the surgery with a fresh complaint.

Controls:- Symptom-free individuals were selected from the same population amongst the family contacts. From the epidemiological point of view the siblings of the index cases from the same age groups were considered to be most suitable for comparison, as they shared the same environmental factors and risks. Because it was quite impossible to obtain materials from perfectly healthy families (no access to them was available) it was necessary to accept the family contacts as the next best controls available. It is appreciated that they do not form perfectly valid controls when an assessment has to be made of the role of the viruses isolated in the aetiology /

FORM 1

SURVEY NO.....

STUDY OF SORE THROAT SYNDROME

in 3-15 Year Olds

Include all cases of "Sore Throat " or those with one or more of the undermentioned clinical findings.

NAME ..... Sex (ring) M.F

ADDRESS ..... Date of Birth .....

SCHOOLS Class .....

West Pilton Primary
St. David's Primary
Craigroyston Secondary
Royston Primary
Royston Nursery

Date first seen..... Duration of illness (days).....

Patient's presenting complaint .....

Patient's attempts at treatment (tick)

Aspirin or equivalent
Antibiotic lozenges (specify)
Other (specify)

Clinical findings (tick)

Pain on swallowing
Tonsillar exudate
Cervical adenopathy
Nasal discharge
Pyrexia
Vomiting
Diarrhoea
Conjunctivitis
Other (specify)

Clinical diagnosis (tick)

Pharyngitis
Follicular tonsillitis
Other (specify)

Treatment (tick)

Non-specific
Other (specify)
Penicillin oral (dose)
Penicillin parenteral (dose)

aetiology of "sore throat". Nevertheless marked differences in the two groups have been observed and the comparisons made are not without value or significance. In many cases the words "healthy siblings" have been substituted for "controls". (Cruickshank 1961, Tyrrell 1963). (M.R.C. Rep. 1965).

Adult contacts:- The adult contacts were also included in the study, but the data obtained from them has been processed separately so that the controls and index cases could be more satisfactorily assessed.

Family:- The term family for all practical purposes has been considered to include all persons living under the same roof and has often included the grandparents, an occasional uncle or aunt and once or twice a boarder.

Clinical and Epidemiological procedures:-

Index cards:- The decision to include a child in the study was made by the physicians at the surgery and a definite protocol was followed. The physicians filled up the details on form 1, and sent it with the throat swab to the laboratory; the information on these forms has provided the sole source of clinical data about the Index cases in this study.

From Index number 235 onwards whole blood specimens were taken on the first visit to the surgery (Acute specimen) and between the second and fourth week after the onset of symptoms (Convalescent specimen). The blood /

blood was collected by the finger prick method on filter paper strips. (Chin et al., 1966).

On the receipt of the specimen and the clinical data the Index case was given a number e.g. I 25, and this number accompanied all the controls and adult contacts associated with this case.

Family specimens:- Within two or three days of the receipt of the specimen from the index case a visit was made by the liaison officer to the family to fill in form 2, and to take the throat swabs of all the available members of the family. Notes were taken of any minor complaints in the family at the time of the visit. The numbering system on form 2 and on specimens from the family was so devised as to identify any particular member of the family. Father and mother were marked as F and M, and brothers and sisters as B1, S2 etc. as they appeared serially on the form. If any of the children in the family later on developed symptoms and visited the surgery he or she was considered a new index case and the family was revisited and the procedure of family visit was repeated.

Follow ups:- When a virus was isolated from the Index case or from any other member of the family a further visit was made by the liaison officer to collect follow up specimens from the entire family in order to determine the persistence of the virus. This was usually between the /

FORM 2. Survey No.

School of I.C.

Class:

Date of G.P. Visit:

Family Name:

Address:

G.P. of I.C:

NAME	DATE	Reln.	Age	I.C.	O.P.S.V.I. T.B.	O.P.S.V.I. T.V.	O.P.S.V.I. N.B.	O.P.S.V.I. Nature	O.P.S.V.I. T	O.P.S.V.I. N	O.P.S.V.I. T.B.	O.P.S.V.I. T.V.	O.P.S.V.I. N.B.	O.P.S.V.I. Nature	O.P.S.V.I. T	O.P.S.V.I. N
	Father															
	Mother															
SCHOOL																
PRESCHOOL																

Form 2

Form used for taking the initial and follow up family specimens for bacteriology and virology.

Reln = Relation I.c. = Index case T.B. = Throat swab bacterial

T.V. = Throat swab virological N.B. = Nose swab bacteriological

O.P.S.V. = Other presumed streptococcal and viral infections

T. = Throat N. = Nose

the 4th and the 5th week.

Laboratory procedures:-

Collection of specimens:-

Initial throat swabs were collected at the surgery with an Exogen disposable sterile swab which was broken into a bijou bottle containing 3 mls. of transport medium. The most suitable type of swab and transport medium had previously been determined experimentally (See page 48 Experimental). The specimens were kept at 4°C till they were collected by the liaison officer in the evening and transported to the laboratory in a thermos flask filled with ice cubes. On reaching the laboratory 300 units of penicillin, 300µg. of streptomycin and 150 units of mycostatin suspended in 0.1 ml. P.B.S. were added to the transport medium containing the specimen. After the addition of the antibiotics the specimens were transferred to the deep freeze at minus 70°C. until tissue cultures were available for inoculation. The specimens from the family were collected by the liaison officer in 3mls. of transport medium and after the addition of the usual amount of antibiotics were kept at -70°C. in the deep freeze.

Virus Isolation techniques:-

The specimens were inoculated in 0.2 ml. amounts into two tissue cultures each of the following cells:-

1. Hep-2 cells (maintained at the Wellcome Laboratory, City Hospital, Edinburgh).

2. /

2. Secondary monkey kidney cells (from the Biological Standards Control Laboratory, M.R.C.).

3. Human embryo lung cells (obtained from the Western General Hospital, Edinburgh).

The Hep-2 and secondary monkey kidney cells were maintained in medium 199 obtained from the Glaxo laboratories and 2% calf serum sodium bicarbonate 4% of a 4.4% solution and penicillin and streptomycin 100 units per ml. were added. (Medium 199 from Glaxo laboratories always contain some amount of antibiotics and sodium bicarbonate). Mycostatin 50 units/ml. was added when their was a mycotic contamination. The human embryo lung cells were maintained in MEM Eagle's medium (Flow laboratories) containing 2% calf serum and 1% Glutamine with penicillin and streptomycin (100 Units and μg/ml) and Mycostatin (50 Units/ml).

All the cultures were rolled at 34°C. They were observed for a visible CPE on alternate days and changed on the fifth day; on the tenth day the cultures were frozen and thawed and a blind passage was carried out into new cultures. Haemadsorption tests were carried out on all monkey kidney cultures on the fifth day of the blind passage with 0.4 per cent. human group "O" red cells and once again before finally discarding the cultures. If any of the cultures showed cytopathogenic effect or a positive haemadsorption test they were frozen, thawed /

thawed and inoculated into more cultures for the propagation of the virus.

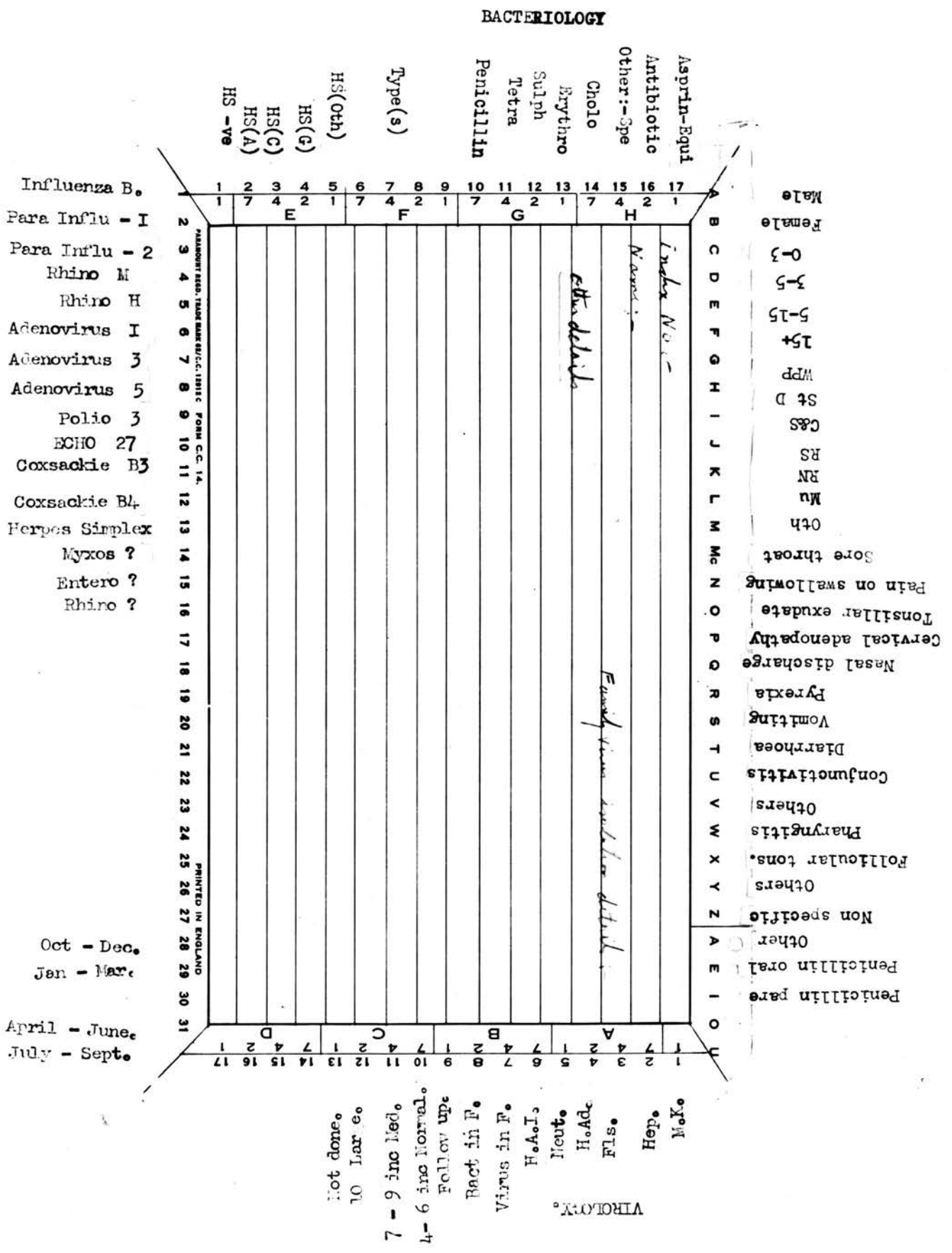
Identification of virus isolates:-

The tube neutralization test as described by Grist et al (1966) was used for the identification of the enteroviruses, adenoviruses and herpes simplex viruses. Virus neutralising sera were obtained from the Standards Division of the Central Public Health Laboratory, Colindale. The haemadsorption inhibition test was used for the identification of influenza and para-influenza viruses. (Vogel and Shelokov 1957). The haemagglutination inhibition test was used for the identification of influenza viruses. Some of the influenza viruses identified by us were sent to Dr. H.G. Pereira of the World Influenza Centre, London for confirmation. Rhinoviruses were characterized by their lability at pH4 and by their ability to grow in monkey kidney and / or human embryo lung cells.

Compilation of results:-

All the details from forms 1 and 2 and the isolation and identification of viruses was transferred to punch cards for final analysis.

Punch card.


BACTERIOLOGY
Aspirin-Equi
Antibiotic
Other:-Spe
Cholo
Erythro
Sulph
Tetra
Penicillin
Type(s)
HS(Oth)
HS(G)
HS(C)
HS(A)
HS -ve
Influenza B.
Para Influ - I
Para Influ - 2
Rhino M
Rhino H
Adenovirus I
Adenovirus 3
Adenovirus 5
Polio 3
ECHO 27
Coxsackie B3
Coxsackie B4
Herpes Simplex
Myxos ?
Entero ?
Rhino ?
Penicillin pare
Penicillin oral
other
Non specific
others
Follicular tons.
Pharyngitis
others
Conjunctivitis
Diarrhoea
Vomiting
Pyrexia
Nasal discharge
Cervical adenopathy
Tonsillar exudate
Pain on swallowing
Sore throat
oth
Mu
RN
RS
C&S
St D
WPP
15+
5-15
3-5
0-3
Female
Male
Oct - Dec.
Jan - Mar.
April - June.
July - Sept.
Not done.
10 Lar e.
7 - 9 inc Med.
4 - 6 inc Normal.
Follow up.
Bact in F.
Virus in F.
H.A.I.
Neut.
H.Ad.
Fls.
Hep.
M.K.
VIROLOGY.
PARAMOUNT REGD. TRADE MARK OR/C.C. 1951 C FORM C.C. 14.
PRINTED IN ENGLAND

Serological Techniques:-

Filter paper techniques for collection of blood:-

Blood was collected on filter paper strips 7mm. wide. The strips were soaked in whole blood obtained by the finger prick method and placed in plain plastic bottles. (Chin et al. 1966).

On reaching the laboratory the stoppers were removed from the bottles and they were left in the laboratory drawer to let the blood dry at room temperature. After drying, a length of the paper equivalent to that soaked by 0.04 ml. of blood was cut into minute pieces and placed in a syringe containing 0.64 ml. of veronal buffered saline. The needle end of the syringe was stoppered by a plastic device and the top covered with an adhesive label. The syringes were kept was 4°C. overnight in the cold room.

After soaking the blood strips overnight, the eluate was forced out with the help of the plunger through the needle into stoppered test tubes and was inactivated at 56°C. for 30 minutes. After inactivation, the liquid was spun in a centrifuge for one hour at 2100 r.p.m. and the supernatant was used as the initial 1:16 dilution of the "serum".

Complement fixation tests

Microtechnique

Complement fixation tests were carried out on acute /

acute and convalescent samples of blood. The "Micro-titrator" unit for serologic microtitration manufactured by "Laboratoriumi Felszerelssek Gyara Budapest" (Takatsy) was used. The disposable trays used for microtitration were manufactured by Linbro Chemical Co.U.S.A. marked IS - MRC - 96.

For the tests the method described by Bradstreet and Taylor (1962) was followed except that 2½ MHD50 of complement were used.

Macrotechnique:-

Macrotechniques were only used for comparison in the initial stages and for confirmation elsewhere. Macro-complement fixation tests have been carried out exactly in the same way as the microtechnique tests except for the volumes which were larger and the trays used were W.H.O. plates.

Amounts used:-

For microtitration 1 volume = 0.025 ml.

for macrotitration 1 volume = 0.1 ml.

Fixation time.

Overnight fixation method at 4°C was used in all complement fixation tests.

Virus antigens used.

The following virus antigens were used in the complement fixation tests.

Influenza A, B and C
Sendai (to cover para-influenza 1)
Adenovirus /

Adenovirus.
Respiratory syncytial virus.
Herpes simplex virus.

Sources of Reagents.

All complement-fixing reagents except the herpes simplex antigen and sheep red cells were obtained from the Standards Laboratory for Serological reagents, Central Public Health Laboratory, Colindale.

The herpes simplex antigen was prepared in GRK cells in the City Hospital Virus Laboratory and the sheep red cells were obtained from Messrs. Burroughs Wellcome Ltd.

EVALUATION OF THE EFFICIENCY OF THE FILTER PAPER METHOD AND THE MICROTECHNIQUE.

On observing the low increases and titres in the index cases using the filter paper eluates and the micro-technique, it was decided to compare the results obtained by this method with the result of complement fixation tests using serum obtained in the usual way.

Source of material

Rabbits after immunisation were used as a source of blood and serum.

Immunisation of rabbits

Three pairs of rabbits were immunised by different routes and different antigens.

First pair:- The first pair of rabbits was immunised with influenza vaccine (Inflac^{R}.) Influenza virus vaccine (inactivated) manufactured by Crookes Laboratories Ltd.

Combination /

Combination of A2/Neth/67/1963. 10,500 H.A. Units and B/Jh6/33/1958/7,000 H.A. Units preserved with Thiomersol 0.01%, plus an adjuvant (oil and Arlacel A).

Two injections were given intradermally 0.5ml. on day Zero and 0.2 ml. on day 80.

Second pair:- The second pair of rabbits was immunised with a single intravenous dose of 0.5 ml. of the above vaccine without adjuvant on day zero.

Third pair:- The third pair of rabbits was infected with a laboratory strain of herpes simplex virus intra-corneally on day zero.

Treatment of serum with 2-Mercaptoethanol.

Equal volumes of 1:8 dilutions of serum and 0.05 M mercaptoethanol (Anderson et al 1964) were kept overnight in stoppered test tubes for 24 hours.

After treatment for 24 hours the contents were transferred into a piece of 8/32" dialysing tube one end of which was tied into a knot, after filling; the other end of the tube was also tied to make a sort of a bag.

The bags were then dialysed in a beaker for 48 hours with frequent changes of buffer (veronal buffer saline).

After 48 hours the contents were transferred to stoppered tubes as 1:16 dilutions and kept at 4°C. till the complement-fixation tests were performed.

A second portion of 1:8 diluted serum was treated similarly using /

using the buffer (VBS) instead of mercaptoethanol. This was used as control serum in the CFTs.

Immunoelectrophoresis:-

Immunoelectrophoresis was carried out on glass slides covered with Oxoid ion agar No. 20, a 1.5% mixture of the agar with the working buffer solution was used for this purpose.

Electrophoresis was carried out at a constant current of 1.5 milliampiers per inch of slide width and a voltage between 90 to 120 volts. For the observation of the passage of albumen fraction the test materials were stained with bromophenol blue. The separation was usually complete after 115 to 120 minutes.

After electrophoresis of the materials goat antiserum to rabbit serum (Hyland Labs) was added to the trough and the slides were left in a cool damp place for the development of the lines of precipitation. After development of the lines of precipitate, the slides were photographed for record.

Statistical methods used:-

A specially developed computer programme was employed for the computation under the supervision of Mr. S.A. Sklaroff. The statistical unit of the University of Edinburgh Department of Social Medicine carried out the computations and the programme was developed by Mr. Sklaroff.

The /

The numbers being small Chi -squared test for statistical significance could not be employed, therefore partitioning of X^2 Chi -squares as described by Maxwell (1961) was employed in most cases, Chi -squared test was only employed in the comparison of results from index cases and the control group.

RESULTS.

CHAPTER I.

Over a period of eighteen months and two weeks between 15th of December 1965 and 29th June 1967, three hundred and ten index cases were examined. All but four were between the ages of infancy and fifteen years. The four cases above fifteen years of age have been excluded from the evaluation of the results leaving 306 index cases as the subject of the study (Hist. 1) (Table 1A).

During the same period 753 healthy persons including 454 healthy siblings of the same age groups as the index cases and 299 adult members of their families were examined. The children of the same age groups have been used for the comparison of results as healthy siblings and the adults are only included in the family studies. (Table IB).

VIRUS ISOLATION

Index cases:-

Viruses were isolated from 45 (14.7% of the 306 index cases (Table IA) and β. haemolytic streptococci were isolated from 79 (25.8%). Both viruses and β. haemolytic streptococci were isolated from nine cases. The double isolations on statistical analysis proved to be according to expectations assuring of independent occurrence and no competition. (Table IB). Thus the number of cases from whom a virus or β. haemolytic streptococcus was isolated was 124 and the total isolation rate /

rate 40.5%.

Age distribution:-

The numbers and positive percentages of the three age groups for the isolation of viruses, β. haemolytic streptococci and combined isolations are given in (Table III), and for viruses only in (Table IV). (Hist 2).

Highest isolation rates for viruses only (12.5% and 4.2% combined) was obtained in 3 to 5 year olds and the highest isolation rate for β. haemolytic streptococci (55.5%) was in the 0 - 3 year olds.

Comparisons with controls according to age groups are also given in these tables.

Seasonal isolation

Seasonal isolations from the index cases are given in (Hist. 1) and have been dealt with individual viruses.

From the 454 healthy siblings belonging to the same age groups as the index cases 31 viruses (6.8%) of the total investigated were isolated from 30 children (one child yielded two viruses) and 32 (7.06%) were positive for one or more β. haemolytic streptococci (Table IA).

Presenting symptoms and diagnoses: (Clinical histogram I and II) (Hist 2)

Out of the 306 index cases 189 were clinically diagnosed as pharyngitis and 94 were diagnosed as follicular tonsillitis (Table IIA). The remaining 23 carried various diagnoses e.g. simple sore throat, scarlet fever, cold, influenza /

influenza, laryngitis, sinusitis, Echo ? infections etc.

Pharyngitis:

From the 189 cases diagnosed as pharyngitis 24 viruses (12.7%) were isolated and 51, (26.9%) were positive for one or more types of β. haemolytic streptococci. Amongst the positive cases both viruses and streptococci were isolated from 6 cases.

Follicular tonsillitis:

Among the 94 cases diagnosed as follicular tonsillitis 17 viruses (18.08%) were isolated and 23 (22.23%) were positive for β. haemolytic streptococci.

Other diagnosis:

Among 23 index cases which were given various other diagnoses 4, (17.4%) were positive for viruses and five (21.74%) were positive for β. haemolytic streptococci.

The diagnoses from which viruses were isolated included Echo ?, laryngitis, scarlet fever and upper respiratory tract infection and the diagnoses of the cases with β. haemolytic streptococci included "sore throat", scarlet fever and sinusitis.

Types of Viruses isolated:-

Index cases:

Amongst the viruses isolated herpes simplex was the most frequent, followed by influenza B virus, enteroviruses, para-influenza viruses, rhinoviruses and adenoviruses (Table V).

Herpes /

Herpes Simplex virus:

Index cases:

Fifteen of the 45 viruses isolated (33.3%) were herpes simplex strains, giving an overall isolation rate of 4.9%.

Three of the isolates were obtained from children between 3 to 5 years and 12 from children between 5 to 15 years of age. Herpes simplex virus was not isolated from infants between 0 to 3 years of age (Virus Table 1).

Seasonal Isolations.

On the seasonal isolation of this virus (Hist. 1 and 3) it was observed that there was only one peak of isolation during the first quarter i.e. between January to March 1966. Seven of the 15 viruses were isolated during this period. The isolation rate decreased after the first peak and during the next quarter of April to June 1966, 3 viruses were isolated and during July to September two viruses were isolated. After September 1966, the isolation rate fell to a steady one isolation per quarter till the end of the study.

Presenting symptoms:

Amongst the 15 positive cases (Clin hist I) 12 had an initial complaint of sore throat. Fourteen complained of pain on swallowing and fourteen showed signs of cervical adenopathy. Pyrexia although associated with /

with the majority of cases was only present in ten cases. Nasal discharge was observed in only 3 cases and tonsillar exudate was present in 5 cases only. Vomiting, diarrhoea, and conjunctivitis were not present in any one of the cases (Virus Table 1).

In four cases the clinical findings included other items than those in form 1.

Case 1. Index 3. Tonsillar exudate, cervical adenopathy, nasal discharge and pyrexia were present. Sore throat, pain on swallowing, vomiting diarrhoea and conjunctivitis absent, the symptoms were summed up as febrile cold and the diagnosis was that of follicular tonsillitis.

Case 2. Index 32. Sore throat, pain on swallowing, cervical adenopathy and pyrexia were present. Tonsillar exudate, nasal discharge, vomiting, diarrhoea and conjunctivitis were absent. The symptoms included cold, pain and headache and the diagnosis was that of follicular tonsillitis.

Case 3. Index 44. Pain on swallowing, tonsillar exudate, cervical adenopathy and pyrexia present. Sore throat, nasal discharge, vomiting, diarrhoea and conjunctivitis absent. The findings included a swelling in the neck and the diagnosis was again that of follicular tonsillitis.

Case 4. Index 152. This was the only case where herpes stomatitis was observed and the positive findings only included pain on swallowing, cervical adenopathy and pyrexia. The diagnosis was made as pharyngitis with a clear indication of clinical herpes simplex.

Diagnosis:-

The clinical diagnosis of the positive cases (Clin. Hist I) were divided into 8 pharyngitis with one case /

case having an additional indication of herpes stomatitis and 7 follicular tonsillitis.

Streptococcal association:

In only 2 of the 15 herpes simplex virus positive cases (Virus Table 1) one or more types of β. haemolytic streptococci were isolated. From one case I79 streptococci belonging to Group A type 4 and 28 were isolated and from another case I139 one streptococcus belonging to Group A type 28 was isolated.

Follow up:

In the follow up of the positive cases and families no viruses were isolated.

Isolations from Controls and Adult Contacts.

Index herpes simplex positives: (Table XII)

Viruses:

Herpes simplex Virus: In only one of the 15 herpes simplex positive index cases (Hist 4) herpes simplex virus was isolated from 3 healthy siblings in a family of nine.

Other viruses: Adenovirus 5. Adenovirus 5 was isolated from the siblings of two herpes simplex positive index cases.

Enteroviruses: Two viruses with the general properties of enteroviruses were isolated from a one year old brother and a 3 year old sister of a boy from whom a herpes simplex virus was isolated.

Streptococci:/

Streptococci:

One β. haemolytic streptococcus group A type 4 was isolated from two siblings of a herpes simplex positive index case. In another case one streptococcus belonging to Groups C or G was isolated from the mother of a herpes simplex positive index case. None of these index cases had a streptococcal association.

Herpes Simplex Virus from the Controls and Adult contacts of negative Herpes Simplex Index-Cases: (Table XII)

Fourteen herpes simplex viruses were isolated from the controls and adult contacts of herpes simplex negative Index-Cases.

Controls: Nine herpes simplex viruses were isolated from the siblings between 0 to 15 years of age.

Adult contacts: Five herpes simplex viruses were isolated from the parents and siblings over 15 years of age.

Seasonal isolation from controls:

Siblings: From January to March 1966, 3 herpes simplex viruses were isolated (Hist 3) from the siblings. During April to June 1966 no herpes simplex viruses were isolated from the siblings. In the quarter between October 1966 to December 1966, there were 4 positive herpes simplex isolations from the siblings. During January to March 1967 again there was no herpes simplex virus isolated from the siblings but one was isolated during the April to June quarter 1967.

Conclusions: (Table IB)

Herpes simplex virus has been isolated from 15 (4.9%) of the Index Cases. This is the highest percentage of any virus isolated from the Index Cases in the present study.

As regard to the significance of this virus in the /

the sore throat syndrome the 15 viruses isolated, form 33.3% of the total isolates.

The significance of these viruses in this syndrome becomes clear on comparing the isolation of these viruses from 306 index-cases and the 454 symptomless siblings the isolation rate was only 2.6% almost half that of the index cases. On the basis of these numbers the contacts should have yielded 0.5 times more viruses than the index cases giving a total of 22.5 viruses but in actual fact only 12 viruses were isolated from the contacts which tabulated to only a yield of about 55% efficiency indicating that more viruses per person were isolated from sore throat cases. (Hist 4).

Influenza B. virus:-

Index Cases:-

The beginning of the study coincided with respiratory tract illness outbreaks in the U.K. from which Influenza B viruses were isolated and this outbreak assumed epidemic proportions in certain parts of Scotland (Circular Virus Unit W.H.O. Geneva 7th March 1966).

Utmost care was taken to exclude the influenza cases from the study. However some of the cases which did not show the influenzal symptoms and fitted in with the clinical requirements of this study filtered in and thus became a part of the study. One point deserves a mention, that when isolation was attempted from the frank influenza /

influenza cases 6 out of 10 showed a positive isolation, a rate much higher than that obtained from the cases included in this study.

Ten, (22.2%) of the 45 viruses isolated during the study have been influenza B viruses giving an over all isolation rate of 3.26%. Eight of these viruses were isolated from children between the ages of 5 to 15 years and 2 from children between 3 to 5 years of age. (Virus Table II, Table V).

Seasonal Isolations:- (Hist 1, 3).

All the influenza B viruses were isolated during one quarter January to March 1966 and particularly between 9th and 25th January, 1966 (Hist. 1). One of the viruses sent to Dr. Pereira of W.H.O. influenza Centre London, along with three other viruses obtained from the siblings was designated by him Influenza B/Scotland/6/66.

Presenting symptoms:-

Seven of the 10 patients from whom influenza B virus was isolated complained of a sore throat (Virus Table II, Clinical histogram 1), nine had cervical adenopathy, seven had pyrexia, 5 had a nasal discharge, 5 pain on swallowing and 4 had tonsillar exudate. Vomiting, diarrhoea and conjunctivitis was absent in all.

In three positive cases the clinical findings included other items in addition to those in form 1.

Case 1. Cervical adenopathy, nasal discharge and pyrexia were /

were the only positive symptoms as included in form 1 but the findings included a rubelliform rash and the patient was diagnosed as ? echo virus infection.

Case 2. Tonsillar exudate, cervical adenopathy, nasal discharge, were the positive symptoms (form 1) but the findings included febrile convulsions. Diagnosis pharyngitis.

Case 3. Tonsillar exudate, cervical adenopathy, nasal discharge and pyrexia, positive (form 1) and the findings included headache and nausea. Diagnosis follicular tonsillitis.

Diagnosis:-

5 of the ten cases were diagnosed as pharyngitis and four were diagnosed as follicular tonsillitis (Virus Table II). One case with a rubelliform rash was diagnosed as ? echo virus infection.

Streptococcal association:-

In 3 of the ten cases positive for influenza B virus, β. haemolytic streptococci were also isolated. The types included 2 Group C or G, and one with Group A, types 8 and 25. (Virus Table II).

Follow up:-

In the follow up of the positive cases and families no viruses were isolated.

Isolation from Controls and adult Contacts:- (Table XII).

Index /

Index influenza B virus positives:

Viruses:-

Influenza B virus. Three influenza B viruses were isolated from the healthy siblings of influenza B virus positive index cases. The virus which was recovered from 27 B_1 was sent to London for confirmation and was designated Flu B/Scot/10/66.

Adult contacts:-

One influenza B virus was isolated from one of the mothers.

Other viruses:

One herpes simplex virus was isolated from the sister of an influenza B virus positive index case.

Streptococci:-

Controls:- β. haemolytic streptococci were isolated from 3 of the siblings of influenza B positive index cases. None of the index cases of these siblings had a streptococcal association. All streptococci belonged to Group A types 5, 12, 2, 4, 3, 13.

Adult contacts:-

One mother yielded streptococci belonging to Group A types 11, 27. The index case had no streptococcal association.

Influenza B Virus from controls and Adult Contacts of negative influenza B virus index-cases.

Controls:- Influenza B virus was isolated from 3 siblings of influenza B virus negative index cases. Two of /

of these isolates were sent for identification and were confirmed by Dr. Pereira as Flu B/Scot/2/66, Flu B/Scot/9/66.

Adult Contacts:- Influenza B virus were isolated from mothers of two influenza B virus negative index cases. One of the index cases was positive for a Myxovirus.

Seasonal Isolation from Controls:-

Between January to March 1966, 6 influenza B viruses were isolated from the siblings. No influenza B virus was isolated before or after this period. (Hist 3).

Conclusions:- (Table IB)

Although influenza B virus was isolated from the index cases during a period when the disease had attained epidemic proportions in parts of Scotland no attempts had been made to include influenza cases in the study. The isolates represent the type of cases in which the symptoms were not clear enough to be included in the influenza cases and presented a milder picture which could only be clinically diagnosed as "sore throat", pharyngitis, follicular tonsillitis or some other form of upper respiratory tract infection. The isolates represent an overall isolation rate of 3.2% during the study which may well be normal for such a study.

If we compute the isolation of influenza B virus in the quarter in which they were isolated and which covered the epidemic period in Scotland mentioned earlier the /

the isolation rate for this quarter was 12.1% which is below that of the general isolation rate for all viruses during the study and far below the isolation rate expected for an epidemic period.

Para-influenza Viruses 1 and 2.

Six para-influenza viruses (1.9%) were isolated during the study from the index-cases. Four of the isolates belonged to type 1 and two belonged to type 2. (Table I, Virus Table III).

Two para-influenza 1 viruses were isolated from children between 3 to 5 years of age and 2 para-influenza 1 and 2 para-influenza 2 were isolated from children between 5 and 15 years of age. (Virus Table III).

Seasonal Isolation.

One para-influenza 1 virus was isolated during the quarter of July to September 1966. One para-influenza 2 and 3 para-influenza1viruses were isolated during October to December 1966, and 1 para-influenza 2 virus was isolated during January to March 1967. (Hist. 1) (Hist 3).

Presenting symptoms.

Four of the 6 patients from whom a para-influenza virus was isolated showed sore throat and pain on swallowing. Three had cervical adenopathy, nasal discharge and pyrexia. One case had conjunctivitis.

Tonsillar exudate and diarrhoea were absent in all cases.

Amongst the other complaints than in form 1:

Case 1 had pain in chest and pain on swallowing and was diagnosed as follicular tonsillitis.

Case 2 had sore throat, pain on swallowing, cervical adenopathy, nasal discharge, pyrexia and conjunctivitis.

Hoarse /

Hoarse voice was the extra sign and was diagnosed as pharyngitis. (Virus Table III).

Diagnosis.

The six cases were diagnosed as follows. Three were diagnosed as pharyngitis, 2 as follicular tonsillitis and 1 as "respiratory tract infection".

Streptococcal association.

One β. haemolytic streptococcus belonging to Group A type 3 was isolated from a para-influenza 1 positive index-case.

Follow ups.

In the follow up of the positive cases and families no viruses were isolated.

Isolations from Controls and adult contacts of positive para-influenza index cases.

Para-influenza Viruses:

Two viruses belonging to para-influenza type 2 were isolated from two siblings of one of the para-influenza 2 positive index-cases.

Other viruses:

Two viruses with the general properties of enterovirus (unidentified) were isolated from two siblings of one of the para-influenza 1 positive cases.

Streptococci:- No β. haemolytic streptococci were isolated from the controls and contacts of para-influenza virus positive index-cases.

Index /

Index para-influenza virus negative:

Controls:- 2 para-influenza 1 viruses were isolated from the siblings of two para-influenza negative index-cases (Table XII).

Seasonal isolation from siblings:

Two para-influenza 1, and 2 para-influenza 2 viruses were isolated from the siblings during a single quarter October to December 1966.

Conclusions.

The isolation of para-influenza viruses during the winter is not uncommon. (Chanock et al 1962, Batatvala et al 1964, 1965, M.R.C. Report 1965). The isolation rate of 1.9% from index-cases is higher than that from the control group (0.8%).

In the absence of any epidemic due to these viruses the present share of 13.3% of the total isolates associated with the sore throat syndrome is substantial.

These viruses in other studies have more commonly been isolated from children with lower respiratory implications than the upper respiratory symptoms. (Chanock et al 1962, Banatvala et al 1964, 1965). Another factor which seems to increase the isolation rate but was missing in the present study is the use of institutionalised population like residential nurseries or creches.

Rhinoviruses:-

Five viruses, (1.6%) isolated from the index-cases were rhinoviruses. Four of the rhinoviruses belonged to the M. types and 1 belonged to the H type. All were isolated from children between 5 to 15 years of age. (Table I). (Virus Table IV).

Seasonal Isolation:

Two of the M rhinoviruses were isolated during January to March 1966 and 2 M rhinoviruses and 1 H rhinovirus were isolated during October to December 1966. (Hist 1). (Hist. 3).

All 5 positive rhinovirus index-cases complained of pain on swallowing. Sore throats, cervical adenopathy and pyrexia were observed in 3 out of 5 cases. Nasal discharge was only present in 2 of the 5 cases. In 3 positive cases the clinical findings included other items than those present in form 1.

Case 1. Pain on swallowing, pyrexia with the additional complaints of headache and observation of inflamed tonsils. The diagnosis was that of adenoidal fever.

Case 2. Pain on swallowing and pyrexia with the additional complaint of acute left otitis media. The diagnosis was that of laryngitis.

Case 3. Sore throat, pain on swallowing, cervical adenopathy and pyrexia with the additional complaint of erythema /

erythema nodosum. The diagnosis was that of pharyngitis.

Diagnosis:- Besides the two diagnosis of adenoidal fever and laryngitis the other three were diagnosed as pharyngitis.

Streptococcal association:

β. haemolytic streptococci Group A were associated with two of the positive index-cases.

Follow up:- In the follow up of the positive index-cases and families no viruses were isolated.

Isolation from Controls and adult contacts.

Index rhinovirus positives:-

Rhinoviruses: No rhinovirus was isolated from any other source than the index-cases.

Other viruses:-

Siblings:- No viruses were isolated from any of the siblings of the rhinovirus index-cases.

Adults:- One influenza B virus was isolated from one of the mothers of the rhinovirus M positive index-cases.

Streptococci:- No streptococci were isolated from the contacts.

Index rhinovirus negatives:-

No rhinoviruses were isolated from any of the controls and contacts.

Conclusions:-

An overall isolation of 1.6% rhinoviruses in a study /

study of sore throats may look very small but agrees with other studies of this type (M.R.C. 1965). The figures of 5 rhinoviruses from a total of 45 isolations represent 11.1% which compares very favourably with the M.R.C. results of a representation of 9.3% in total virus isolates of 86 from their 376 sore throat cases and with an overall percentage of isolation of 2.2.

However if organ cultures were to be employed then the chances of a higher isolation rate for rhinoviruses would have improved considerably (Higgins 1966a).

On the other hand a higher isolation rate of 2.9% and a percentage of 37.2 of a total of 59 viruses was obtained in a study by Pereira et al., (1967) in primary school children but in this study the children included were suffering with "cold" or other minor respiratory ailments and were swabbed in the school.

Adenoviruses:-

Two adenoviruses (0.65%) were isolated during this study. One adenovirus 1 was isolated from a child of 0 - 3 years and one adenovirus 3 was isolated from a child between 3 to 15 years of age. (Table I, Virus Table V).

Seasonal Isolation:- Adenovirus 1 was isolated during April to July 1966 and adenovirus 3 was isolated during January to March 1967. (Hist. 1). (Hist 3.)

Signs and symptoms:-

In /

In the one case from which data is available, sore throat, pain on swallowing, cervical adenopathy and pyrexia were present.

Diagnosis:-

In I276 Adenovirus 3 the diagnosis of pharyngitis was made.

Streptococcal association:-

No haemolytic streptococci were isolated from either of the cases.

Isolation from Controls and adult contacts.

Index Adenoviruses positive:-

Viruses:- No viruses were isolated from contacts.

Streptococci:- No β. haemolytic streptococci were isolated from the contacts.

Index Adenoviruses negative:-

Siblings:-

Two adenoviruses belonging to type 5 were isolated from the siblings of two index-cases from whom herpes simplex virus were isolated. One adenovirus type 3 was isolated from a child whose index-case was negative for virus.

Adults:- No adenoviruses were isolated from the adults.

Seasonal Isolation:-

Two adenovirus 5 were isolated during April to June 1966 and adenovirus 3 was isolated between January to March 1967.

Conclusions:-

An /

An overall isolation rate of 0.65% has been achieved from the adenoviruses with a representation of 4.4% of total virus isolates. This agrees with (Higgins et al., 1966) results of isolations of adenovirus from sore throats representing about 5% of the total isolates, and the primary school study of (Pereira et al., 1967) when the sore throats did not yield a single adenovirus. These isolation results do not agree with the (M.R.C. report 1965) findings where they isolated a total of 31 adenoviruses from 376 sore throat cases an over all isolation rate of 8.2% and a representation of 34.8% in the total isolation of 86 viruses. It is possible that certain factors especially present in some areas favour the occurrence and isolation of adenoviruses and these have affected the results of the nationwide M.R.C. study.

Perhaps the method gaining acceptance in the isolation and recognition of adenoviruses of performing complement fixation tests on the culture fluids would have benefited the isolation rates of these viruses in the present study.

Enteroviruses:-

Index cases:-

Seven, (2.2%) enteroviruses were isolated during the study. Four were identified in the laboratory by neutralisation tests and belonged to Echo 27 (one) Cox. B_3 (two) and Cox. B_4 (one). Three proved to be difficult to /

to identify in the laboratory with the existing sera and were sent to Glasgow. Six were isolated from children between 5 - 15 and one from a child between 3 - 5 years of age. (Hist. 1 Virus Table VI).

Seasonal Isolations:- (Hist 1, Hist. 3).

One Echo 27 and two others (entero-like but unidentified) were isolated during April to June 1966. One enterolike virus was isolated during July to September 1966.

One Coxsackie B_4 and one Coxsackie B_3 were isolated during January to March 1967 and one Coxsackie B_3 virus was isolated during April to July 1967 (Hist. 1).

Presenting symptoms.

This was the most remarkable group as regards to signs and symptoms. (Clin. Hist. 2). (Virus Table VI). All the seven had sore throats, cervical adenopathy and pyrexia. Six out of seven had pain on swallowing, and four out of seven had a tonsillar exudate. One complained of a sore head. Nasal discharge, vomiting, diarrhoea and conjunctivitis were absent in all.

Diagnosis:-

Out of the seven three were diagnosed as pharyngitis and four as follicular tonsillitis.

Streptococcal association:-

Only one case I 135 had a haemolytic streptococcus belonging to Group C or G.

Follow /

Follow ups:-

In the follow up of the positive cases and families no viruses were isolated.

Isolation from Controls and adult Contacts. (Table XII).

Index enterovirus positive:-

Viruses:- Only one herpes simplex virus was isolated from the mother of I 135 and I 137 who were siblings.

Streptococci:- No haemolytic streptococci were isolated from the contacts.

Isolation from controls and adult contacts of negative enterovirus index cases.

Siblings:- Two viruses with the general properties of enteroviruses were isolated from two siblings of a herpes simplex positive index case.

One poliomyelitis virus type 3 was isolated from a 3 year old child. On enquiring from the general practice it was found out that the child had recently had a polio vaccine. The index case of this child was virus negative.

Two entero-like viruses (sent to Glasgow) were isolated from two brothers aged 2 years and 3 years of an index case who was para-influenza 1 positive.

One poliomyelitis virus type 1 was isolated from a 9 month old brother of a virus negative index-case on enquiry the child proved to have taken a polio vaccine prior to the taking of the sample.

Adults:- One entero-like virus was isolated from a 17 year /

year old sister of a virus negative index case.

Seasonal isolation from controls and contacts.

Controls:-

Three unidentified entero-like viruses were isolated during January and March 1966. During this period no enteroviruses were isolated from the index cases.

During April to June 1966 one poliomyelitis virus 3 was isolated from a child.

Two more entero-like viruses (sent to Glasgow) were isolated during October to December 1966. One poliomyelitis virus 1 was isolated from a child during January to March 1967.

No enteroviruses were isolated from the contacts during April 1967 to June 1967.

Conclusions:-

An over all isolation rate of 2.2% has been achieved for enteroviruses in this study representing 15.5% of the total virus isolates. These results agree with the results of Higgins et al (1966) when their enteroviruses represented about 15% of the total virus isolates.

The results do not agree with the isolation rate of the M.R.C. report (1965) where they achieved an isolation rate of 8.7% for enteroviruses in their sore throat cases and a representation of 37% of the total isolates. /

isolates. This difference is not explainable in ordinary terms and may be due to the wide spread representation of communities and localities in the M.R.C. study.

The isolation results however are better than those achieved by Pereira et al (1967) when they could obtain only three enteroviruses out of the 782 illnesses studied, but once again they were dealing with a different set of symptoms, and symptoms may be very important in the case of enteroviruses isolation as represented by the present results when seven out of seven isolates were obtained from patients with sore throats, cervical adenopathy and pyrexia.

CHAPTER II

FAMILY STUDY

In an effort to follow the distribution of viruses inside the families a total of 753 throat swabs from healthy siblings, parents and other adult members of the family were examined in the laboratory.

The specimens represented as many members of the family as were available at the time of the liaison officer's visit just after the index case was reported.

Altogether 247 families took part in the survey. The total isolation rate of viruses based on all age groups in the families was 5.3% and β. haemolytic streptococci were isolated from 5.1% of the family members.

Viruses isolated:-

Generally the viruses isolated in the families were similar to those isolated in the index cases except that in enteroviruses, poliovirus 1 and poliovirus 3, were isolated in the families while in index cases this group was represented by Coxsackie B_3, Coxsackie B_4 and Echo 27 virus. The general nature of enterovirus yet to be identified was similar. No rhinoviruses were isolated in the families. In the case of adenoviruses one additional type, adenovirus 5 was isolated from two children. Only one child visited the surgery inside two weeks and on the second visit an Echo 27 virus was isolated. In only one case herpes simplex virus was isolated twice, once /

once in the 4th week and once in the 36th week. In only one case an influenza B virus was isolated from a mother and an index case otherwise the viruses appeared independently and without any signs of cross-infection from adults to children and vice versa.

Apparent and inapparent infections:-

In the survey of ratio of apparent to inapparent infections in the families β. haemolytic streptococci seemed to be more often isolated from the (inapparent) healthy siblings than the viruses. (Table III). These organisms have been isolated 12 times in the survey from more than one member of the family. Four times the organisms have been shared between the index case and 1 sibling, 3 times they have been shared between the index cases and their siblings and once they have been shared between the index case and two siblings. In the absence of streptococci from the index case they have been isolated 3 times from two siblings, and once, from 3 siblings.

Amongst the viruses, Influenza B virus was isolated 5 times from more than one member of the family. Three times this virus was shared between the index case and one more sibling. Once it was isolated from three siblings of an influenza negative index case and once it was shared by a mother and the index case.

Only twice in the study the enteroviruses were shared by two members of the family, twice they were isolated /

isolated from two siblings when the index case was entero-virus negative.

Herpes simplex virus was isolated only once from an index case and 3 siblings and para-influenza virus was isolated once from two siblings and the index case. These results indicate a very low rate of cross-infection inside the family as well as low rate of cross-infection of only 5.2% in the 247 families who took part as a unit in the survey.

Families with single and multiple episodes of sore throat.

The three hundred and six index cases were distributed in 247 families. Forty seven families had multiple episodes of an average of 2.25 episodes per family and shared 106 index cases amongst them. One hundred and seventy five families had one episode each. Other 25 families did not take part in the family survey.

Virus and streptococcal isolation:-

Single episode families:- (Table XIII).

Twenty two viruses, (12.5%) and 55 β. haemolytic streptococci (31.4%) were isolated from 175 index cases belonging to these families.

Multiple episode families:-

Eighteen viruses (16.9%) and 22 β. haemolytic streptococci, (20.7%) were isolated from 106 index cases belonging to these families.

Absent families:-

Five /

Five viruses (20%) and 2 β. haemolytic streptococci (8.0%) were isolated from these 25 index cases.

Comparison.

On statistically comparing the isolation result of viruses and β. haemolytic streptococci between the single and multiple episode families it was found that the higher isolation rate of viruses in multiple episode families and β. haemolytic streptococci in the single episode families were both statistically significant (Table XIII) and not a chance occurrence.

On consulting the data cards for the multiple and single visit families no other socio economical factors were found that could give a possible answer to the occurrence of multiple episodes in certain families and single in others than that in the multiple episode families the ratio of medium and large families was higher than the single episode families. Detailed study of this aspect could not be carried out because of the lack of facilities.

CHAPTER III

SEROLOGICAL INVESTIGATIONS.

Complement Fixation tests.

Complement fixation tests were carried out on the acute and convalescent specimens of 34 Index cases, using filter paper dried blood and the microtechnique.

Results.

Ten children showed a fourfold or greater increase in titre against one or more viruses, and one child from whom a herpes simplex virus was isolated showed a specific antibody rise from not detectable to 1/16.

A positive serological diagnosis of 32.3% was obtained in these patients as opposed to the positive virus isolation findings of 14.7%. (Table VI).

Clinical findings:-

Clinical findings of the serologically positive cases have been presented in (Table VII). These findings indicate a severity of symptoms which is comparable with that of all the cases from whom viruses were isolated.

If pyrexia is used as a measure of severity then 100 per cent. of the children from whom an enterovirus was isolated and 73 per cent of cases with serological evidence of infection had severe illnesses. With virus isolations other than enteroviruses fever was present as follows:-

Influenza B virus 70%, Herpes Simplex Virus 66%, Rhinoviruses 60%, Para-influenza Viruses 50% and Adenovirus /

Adenovirus 50%.

The above comparison suggests that in all the serologically positive cases virus infection occurred and but for the serological investigation the proof would have been missed completely.

Conclusions:-

Positive serological findings by means of macro complement fixation tests have often been used to supplement the results of virus isolations in virological investigations. Usually the incidence of positive finding is higher in serological findings than in virus isolations.

The present findings indicate that by using micro-complement fixation tests and dried blood on filter paper, similar results to the routine complement fixation tests can be achieved with a great saving of time, trauma and valuable materials.

As a method of performing serological investigations in children especially outside hospital the present technique has proved itself to be greatly valuable.

Evaluation of the efficiency of the filter paper method using micro technique:-

The results of complement fixation tests performed both by the Macro and Micro techniques in the planning stages showed it difficult to interpret the dried blood results in Macrotechnique. It was decided therefore to use only the micro technique.

Experiment.

Initial complement fixation tests with dried blood and serum:-

On the initial testing of dried blood and serum from six rabbits immunised in the laboratory it was found out that the results with filter paper dried blood were usually lower than those of serum (Table IX).

Loss of titre with filter paper dried blood.

There were two possibilities for this difference in the reactivity of the two materials.

1. A specific mechanical adherence of some of the 19S globulin molecules to the paper pulp, due to its larger molecules.

2. A general mechanical retention of the proteins of the blood by the filter paper.

Testing the hypothesis of specific adherence of 19S globulin:-

There have been reports that the 19S component is rendered inactive by the treatment with mercaptoethanol. This has been shown to be so in agglutination and precipitation tests (Bauer and Stavitsky 1961), as a general decrease /

decrease in antibody activity (Bellanti et al 1962), in complement fixation tests by Anderson et al (1964), in precipitating and neutralising activity by Cowan and Trautman (1965) and agglutination by Tonder and Natvig (1967).

To find out whether it is the 19S globulin fraction that is lost in the filter paper dried blood, it was decided to treat the serum with mercaptoethanol and then compare the results of CFTs with the filter paper dried and control specimens.

Comparison of CFTs with dried blood, serum and Mercaptoethanol treated serum.

On the treatment of serum with mercaptoethanol the CFT results showed differences in the action of mercaptoethanol on the serum of the three sets of rabbits depending upon the route of immunisation (Table VIII).

1st Pair:- Immunised with influenza vaccines plus adjuvant, Dosage 0.5ml intradermally on Day Zero, 0.2ml intradermally on day 80th.

The CFT titres of these two rabbits (1 and 2), showed initial low titres in serum and dried blood from preimmunisation bleeding on day zero. These titres were not grossly affected by the treatment with mercaptoethanol.

However, the titres from the 8th bleeding on day 88 and 9th bleeding on day 92 and after the 2nd injection were considerably high and were reduced to 1:16 after the mercaptoethanol treatment.

This on the one hand indicated the production of 19S gamma antibody after the 2nd injection and on the other hand showed that the lowering of titres in the filter paper method is not due to the inactivation or specific mechanical adherence of the 19S gamma molecules to the filter paper.

2nd Pair:- Was immunised with a single dose of influenza vaccine 0.5 ml. on day zero. The tires of these two rabbits have shown different reactions after treatment with mercaptoethanol.

In /

In rabbit 3 with initial preimmunisation titres of less than 16 in all the three specimens and final bleeding (92nd day) titres rising to the highest of 512, mercaptoethanol treatment of the serum reduced the titres considerably but not to the extent of rabbit one and two indicating the presence of a mixture of antibodies in the form of 7S and 19S globulins. In rabbit 4 however the immunisation did not seem to have any effect and the titres did not increase up to the 8th bleeding (88th day). But the mercaptoethanol treatment did have a little effect on the serum after immunisation that it reduced the titre by two folds again indicating a mixed antibody presence of 7S and 19S globulins.

3rd Pair:- Infected with herpes simplex virus intracorneally on day Zero.

In these rabbits (5 and 6) the treatment of serum with mercaptoethanol did not produce any change in titres of both bleedings i.e. day zero preimmunisation and day 92nd after immunisation. Indicating the presence of 7S gamma antibody alone which could withstand the mercaptoethanol treatment.

Conclusions:- The results as represented by the complement fixation test titres of the three materials (filter paper eluates of dried blood, whole serum and mercaptoethanol treated serum) do not justify the idea of any specific reaction of filter paper material with the 19S gamma fraction /

fraction of the antibody based upon its molecular size.

The loss of antibody activity in filter paper dried blood seems to be related to the problems of using very small quantities of materials and their repeated handling during drying, elution, inactivation and performing the complement fixation tests.

This could only be termed as a loss of material due to handling or a general mechanical hindrance of serum proteins by the filter paper.

If the existence of this loss is kept in mind at the time of performing the tests then the interpretation of results will become much easier as always room will be left for the factor. Further comments on these results will be made in the discussion.

Studies using immunoelectrophoresis.

The purpose of carrying out immunoelectrophoresis was to determine what qualitative changes were present in the serum proteins specially in the globulin fraction after the different treatments, and whether these changes could be correlated with the complement fixation test findings.

It is emphasised that immunoelectrophoresis as used in this experiment is a qualitative and not a quantitative method of examination. However it can yield valuable results and an indication of changes in globulin concentration is reflected by the length of the precipitation arc, its closeness to the trough and the width of the precipitate and these were the provisios in mind at the time of conducting the experiment.

Immunoelectrophoresis.

Immunoelectrophoresis on rabbit sera before and after immunisation.

The antigens used were eluates from filter paper dried blood, whole dialysed serum and mercaptoethanol treated serum. These were developed against goat antiserum to rabbit serum.

Rabbit 1 Pre-inoculation:- (Fig. 1A).

The eluate from filter paper contained so little protein that no bands of precipitate developed.

The dialysed serum gave a clearly defined arc which was much reduced by the mercaptoethanol treatment.

After /

After immunisation:- (Fig 1B).

After immunisation the globulin line can be detected in the filter paper eluate sample but this has a reduced intensity when compared with the dialysed serum. In dialysed serum sample the arc is notably compact and dense indicating that this sample contains much more gamma globulin than the eluate. Similarly when mercaptoethanol treated serum sample is compared with the dialysed sample it it found that the arc is much reduced in intensity and is diffused indicating a loss in the gamma globulin fraction after treatment.

Rabbit 4:- Pre-inoculation:- (Fig 2A).

With the filter paper eluate the arc of precipitation is very thin indicating a lower protein content. The arc in the dialysed serum sample is very sharp but became a little bit hazy after the mercaptoethanol treatment; it did not change much otherwise.

After immunisation:- (Fig 2B).

After immunisation the arc in filter paper eluate sample became somewhat thicker indicating a change in the original pre-immunisation protein content. The arc of precipitation in the dialysed serum specimen did not change much, and it remained as sharp as in the pre-immunisation specimen. However the arc in mercaptoethanol treated sample became fainter and hazier than both the other samples indicating a loss of gamma globulin contents.

Rabbit /

Rabbit 5:- Preinfection:- (Fig. 3A)

With all the three samples the arcs developed with minor differences in intensity and proximity to the troughs indicating small differences in the protein contents of the three samples.

After immunisation:- (Fig. 3B)

All the three arcs showed a marked increase in intensity and sharpness over the pre immunisation samples indicating a marked increase in the gamma globulin contents of the samples.

Conclusions:-

A consistent finding has been that the eluates from filter paper and sera after mercaptoethanol treatment have given poorer precipitation arcs than the whole serum.

This is suggestive evidence that the eluates from filter paper and mercaptoethanol treated sera show a loss of sensitivity as compared with the whole serum.

It is noteworthy that in all cases the precipitation obtained in the case of eluates and mercaptoethanol treated sera was increased after immunisation, and this was confirmed by increased complement fixation titres.

The arcs produced were formed unusually near the well because in this particular set of experiments the electrophoresis was carried out with a low voltage. However, I confirmed that these arcs were in fact gamma globulin /

globulin lines by the use of a specific anti gamma serum.

The goat antiserum showed the arcs to be those of gamma globulin, but the antiserum was not potent enough to allow the observation of changes in alpha and beta globulins.

PART IV

DISCUSSION.

Many sore throats are mild and it is only necessary for the patient to make a single visit to the practitioner's surgery. They do not require hospitalisation and usually respond to simple remedies. They occur nevertheless so frequently that they are very common experiences in urban communities.

The present study has been directed towards a better understanding of this problem in children. It is well established that besides pharyngitis and follicular tonsillitis the complaint of sore throat can occur and can be recognised in a variety of other clinical conditions. The diagnosis may be difficult in infants and small children. Children between 0 - 15 years from a general practice were used as index-cases (with sore throats) to represent a sample of the ordinary child population of a particular area of Edinburgh.

Siblings of the same age groups were used for comparison. Adult and juveniles over the age of 15 in the family were included for studies within the family.

There have been a sizeable number of studies in various groups of the population in Britain and elsewhere in which the workers have tried to study the problem of Acute and Upper respiratory infections.

Studies on the isolation of respiratory pathogens in families have been conducted by several workers, (Dingle et /

et al 1953, Badger et al 1953, Wenner et al 1957, Brimblecombe et al 1958, Jordan et al 1958, Valadian et al 1961, Philip et al 1961, Robinson et al 1962, etc).

These studies have mainly been concerned with the aetiology of Acute Respiratory Disease and quite often have included the lower respiratory lesions in adults as well as in children. Studies of this type are not directly comparable with the present observations.

Other studies that have been conducted on the isolation of pathogens from respiratory tract infections have usually employed hospitalised children or have been conducted in residential nurseries, boarding schools and University students hostels. Most of these populations have either been static in their surroundings with a high degree of cross-infection or have been suffering from more severe illnesses than sore throats. Many patients in these studies were hospitalised and were thus disqualified from the comparison with the present study.

(Kendall et al 1957, Morrison et al 1957, Graham et al 1958, Evans et al 1958, Grieble et al 1958, Chanock et al 1959, Gardner et al 1960, Hobson and Schild 1960, Lewis et al 1961, Hilleman et al 1962, Kendall et al 1962, McDonald et al 1962, Sutton 1962, Connelly and Hamre 1964, Potter and Shedden 1963, Tobin 1963, Holzel et al 1963, Wulff et al 1964a, Gardner et al 1964, Clarke et al 1964, Urquhart et al 1965, Holzel et al 1965, Aitken et al 1967).

The /

The group of studies, some aspects of which are directly comparable with the present study, are those carried out in general practice in this country or America and which included a sizeable proportion of infant and child population. (Kendall et al 1960, Higgins et al 1963, Banatvala et al 1964, Higgins et al 1964, The Medical Research Council Report 1965, Banatvala et al 1965, Higgins et al 1966 and Glazen et al 1967).

Initial experiments.

Selection of swabs and transport medium.

Much of the success in the virus isolation depends on the initial transportation, inoculation and storage of the material. Transport medium and storage conditions are therefore of prime importance in the present type of survey.

Selection of swabs.

The type of swabs used are mostly not even mentioned in the literature and up to the time of this study this point was considered to be unimportant in the isolation procedure for viruses. In September 1965 Mair (P.H.L.S Tech. 65/12) reported the inhibitory effect of certain swabs (Johnson's) on viruses. The above report was in mind when the present study was being planned and it was decided to study the types of swabs available in combination with the variations of the transport medium and conditions of transport desirable for the study.

Three /

Three types of swabs were available for use in the study.

1. Johnson's Gamma irradiated sterile swabs.
2. Exogen sterile disposable swabs.
3. Surgical swabs prepared in the Wellcome Laboratory, City Hospital, Edinburgh.

Two of these swabs were mentioned in Mair's report and the third was the local swab daily used in the laboratory.

Exogen swabs used with bovine serum albumen in transport medium gave the best chances for survival of the viruses under conditions expected to pertain during the survey. (4°C for 6 hours and storage at -70°C for 24 hours or more).

The experiment was conducted with an open mind as to the conditions of transport, so that if the optimal conditions were not available for all combinations of swabs and transport media, the alternative conditions which gave the best results could be used. The results indicate that Exogen swabs gave good results in both transport media but the results with bovine serum albumen were far better than calf serum for all the viruses.

Selection of Transport medium.

Three types of materials have generally been used as transport media. Balanced salt solutions like Hanks' and Earles and Medium 199 as used by the British and Australian /

Australian workers, nutrient broth and veal infusion broth which seem to be preferred by American authors. To the basic salt solutions workers have added additional proteins and sodium bicarbonate. Various combinations of antibiotics had been used and the temperature of storage has varied from -10° to -70°C.

Graham et al (1958) used Hanks' solution with 1% gelatin and the storage temperature of -10°C while in the same year Evans (1958) used balanced saline solution and 2.5% animal serum with -20°C as the storage temperature. Holland et al (1960) used Hanks' solution with 0.5% lactalbumin hydrolyzate and kept it in flasks of "Cardice". Gardner et al (1960) did not use any added proteins in Hanks' solution but included antibiotics and transported the material on "Cardice" and the same method was used by Kendall et al (1960).

Tyrrell (1963) approved the use of balanced salt solution or medium 199 but recommended the use of 50% broth or 2% bovine plasma albumin and freezing the specimen to -70°C. Pereira et al (1963) used Hanks' solution with 0.5% lactalbumin hydrolyzate and 0.02% sodium bicarbonate but used the storage temperature of -65°C and Higgins et al (1963) used Earle's solution and 0.5% of lactalbumin hydrolyzate and sodium bicarbonate. Urquhart et al (1965) used Hanks' solution with 2% inactivated /

inactivated horse serum and antibiotics and used the storage temperature of -70°C.

The Medical Research Council working party used a combination of Hanks' solution with 0.2% bovine plasma albumin chilled in ice and Pereira et al in (1967) have again used Hanks' solution with 0.2% bovine serum albumin and storage temperature of -70°C.

Medium 199 has been used with added antibiotics by Lewis et al (1961) and Banatvala et al (1965) while 199 with additional proteins as suggested by Tyrrell has been used by Aitken et al (1967) with 1% bovine serum albumin and sodium bicarbonate 5% of a 4.4% solution, antibiotics, transported on ice and stored at -70°C.

The American workers who have preferred broths to salt solutions are Robinson et al (1962) who used nutrient broth and froze the samples to -70°C. Hilleman and others (1962) used Veal infusion broth and froze the samples while Wulff and colleagues (1964a) have used infusion broth with added antibiotics. Probably Tyrrell brings about a compromise in the use of transport media when he suggested the use of 50% broth or 2% bovine plasma albumen.

Unfortunately most of the workers have made little comment on the importance of the transport medium they used. The choice of 1% bovine albumen in medium 199 with 5% of 4.4% solution of sodium bicarbonate and antibiotics /

antibiotics in this study was based upon the results of the initial experiments.

Conditions of Transport.

The conditions of collection directed by the nature of this study were, holding the sample at 4°C for 6 hours and then transport to storage at -70°C for 24 hours or more. Other physical conditions were also tested.

1. Storage at room temperature for 6 hours.
2. 4°C for 6 hours.
3. 4°C for 24 hours.
4. Room temperature for 24 hours.
5. At 4°C for 6 hours and -70°C for 24 hours or more.

Although we were aware of the usefulness of direct inoculation of tissue culture tubes, (Tobin 1963), the limited facilities of the study did not permit the use of this method. Another consideration that was in mind was that by using the direct inoculation method we should be only increasing the possibilities for isolating the respiratory syncytial virus. This virus had, however, proved to be more commonly associated with lower respiratory tract infections in children, the "Sore throats" being used as clinical criteria the results may not be seriously effected.

Investigation of the antiviral activity of calf serum and bovine serum albumen.

In November 1967 the matter of lower isolation rates in general for para-influenza viruses was discussed between Dr. /

Dr. Moffat and Dr. Tyrrell. It was suggested by Dr. Tyrrell that if antibodies and other inhibitory substances present in tissue culture media are avoided a higher isolation rate may be achieved. Monkey kidney tissue cultures maintained in Eagles medium without calf serum were mentioned as useful.

In the present study calf serum was used in the maintainance medium and bovine serum albumen was a part of the transport medium. Experiments were designed (a) to test the survival of three viruses including para-influenza 1 virus after coming in contact with bovine serum albumen, calf serum and Dulbecco A. solutions (b) to test the growth of para-influenza 1 virus in monkey kidney cells maintained on either calf serum and 199 (as used in the study) or on Eagles medium without calf serum.

As indicated by the results bovine serum albumen once again proved to be a better transporting agent than calf serum or Dulbecco A.

From this work it is seen that the para-influenza 1 virus is not inhibited by calf serum as measured by the production of haemadsorption effects in its host cells. In the absence of calf serum however and under the same conditions, the virus produces a high degree of haemagglutination.

Thus calf serum although not hindering the early phases of multiplication of the para-influenza 1 virus may prevent /

prevent the final maturation of the virus and interfere with its release from the host cell.

These results are in a way very similar to those observed by Stern and Tippet (1963) while testing the growth of Influenza A virus at 37°C and 33°C. At 37°C which proved not to be a suitable temperature for growth there was haemadsorption but at the more suitable temperature of 33°C there was obvious cytopathic effect.

The indications are clear as to the desirability of a serum free maintainance medium for para-influenza viruses.

Isolation results.

Kendall et al (1960) described the results of their survey in a general practice. This is the earliest report of a survey of acute respiratory infections in a general practice in which virus isolations were also attempted.

This study to a large extent represented the same age groups as are the subject of the present study. The aim of these authors was not predetermined; they started with rather low expectations with only HeLa cells as their means for detecting the respiratory viruses. In this way they were lucky that a twelve week epidemic (recognised in retrospect) of febrile pharyngitis occurred and gave them remarkable isolation rates of 16.4% for viruses and 12.3% for streptococcus pyogenes.

As /

As indicated by their results and admitted in the discussion of their paper, but for the epidemic of Coxsackie B_3 virus they might have ended up with only 2.7% isolation for the adenoviruses. With the lack of tissue culture facilities in their study they were certainly hampered.

Another finding which came about in this study was the occurrence of secondary febrile illnesses in Coxsackie B positive families. This has not been observed in the group of healthy siblings in the present study although a similar pattern exists in the control group of another local practice. (Brown et al unpublished).

The lack of isolation of viruses from children with other than febrile respiratory infection has been used by Kendall et al to emphasise the presence of Cox. B_3 virus in febrile pharyngitis.

It would be a mistake to compare the results of the present study with the work of Kendall et al because more types of cells have been used in the present study and the laboratory techniques have improved a great deal since 1960. The only points to be emphasised here are the low isolation rates of viruses in dealing with a population of child volunteers which is in no immediate need for hospitalization. The isolation rate for adenoviruses in comparable circumstances in our study was 0.65% as compared with 2.7% of Kendall and colleagues. These /

These averages could possibly be well within the range of isolation for these viruses in the non-epidemic periods.

The isolation rates of β. haemolytic streptococci of (25.8%) in the present study is definitely higher than that of Kendall and colleagues (12.3%); these figures may represent the respective incidence of these organisms in febrile pharyngitis in the two studies.

The next study that was comparable from the point of view of laboratory technique and the representation of children between 0 to 15 years of age came from Higgins et al (1963). They used a comparable set of host cells to the present study e.g. Secondary monkey kidney cells, Bristol HeLa cells and human embryo kidney cells. The temperatures they used for the cells differed from the present study in that they rolled the monkey kidney cells at 37°C and did not use serum for their maintainance medium for these cells. Their HeLa cells were kept stationary at 36°C.

In the present study the corresponding cells have been secondary monkey kidney cells, but HEP.2 cells have been used instead of HeLa cells and human embryo lung cells were used instead of human embryo kidney cells. All the cell cultures in the present study were rolled at 34°C and calf serum was used in the maintainance medium for monkey kidney cells.

Among 428 specimens examined by Higgins et al, 189 derived from the same age group as in the present study,

0 to 15 years, but the clinical criteria differed in the two studies. In the present study "sore throat" was used as the index symptom but the cases examined by Higgins et al included sore throats, pharyngitis as well as colds and lower respiratory illnesses. As in the present study the list of viruses isolated by Higgins et al included influenza B virus, para-influenza 1 and 2 viruses, herpes simplex virus and adenoviruses, rhinoviruses H and M and also β haemolytic streptococci. It differs however, in the isolation of R.S. virus as well as the nature of entero-viruses which included polio virus 1 and 2, agents that did not occur in index cases in this survey.

Comparison of the isolation rates of various viruses in the two studies signifies the importance of the differences. The virus isolation rate of 24.8% is higher in Higgins et al's study by about 10%. This might be firstly because they were using a wider set of clinical entities and secondly because their techniques (either accidentally or deliberately (not mentioned)) differed from those used in this survey, for example, they used direct inoculation of M.K. and HeLa cells without freezing the specimen to facilitate the isolation of R.S. virus and did not use any serum for the maintainance of M.K. cell cultures. This might have improved the isolation rates for myxoviruses. Two striking differences which have no explanation at this stage are the very low isolation /

isolation of 0.52% for herpes simplex virus and 5.2% for β. haemolytic streptococci. The corresponding isolations of the last two agents are quite high in the present study in fact they are numerically the most important isolates being 4.9% and 25.8% respectively.

One probable explanation is that our criteria for the selection of cases were somewhat biased in favour of streptococci; but the higher isolation rates of herpes simplex virus still requires an explanation.

However in subsequent observations by Higgins et al (1964, 1966) their isolation rate for herpes simplex virus reached 3.4%. These authors isolated many more strains of R.S. and other viruses but their isolation rate for streptococci was a little less than one fifth of that of the present survey. Higgins et al's isolation rate of 4.5% viruses from the healthy subjects compares favourably with the present study's 6.8% in the healthy siblings.

Banatvala et al in (1964) reported the isolation of para-influenza viruses from children in a general practice in Cambridge. Important facts about this report is that proof of para-influenza virus infection was obtained in 18% of the cases in all age groups and that the involvement of lower respiratory tract was observed. The question of total numbers of patients in various age groups is left for the readers to compute but the fact that the study represented an outbreak has been accepted.

In 1964 Higgins et al published their results on the /

the isolations made from acute respiratory cases during 1963 in a general practice. The changes that were made in the original procedures were the use of 33°C as the incubation temperature for M.K. cells and the use of suckling mice for coxsackie A viruses. Another important factor introduced in this study was the use of three clinical categories of illness namely, cold, sore throats, and flu like illness.

The isolation pattern of organisms from sore throats in children between 0 - 15 years and in general had changed from the earlier part of the study, viruses were isolated from 23.4% of the sore throat cases between 0 - 15 years of age and β. haemolytic streptococci isolations increased considerably in this age group in sore throats to 25.4% thus bringing the total isolation rate for sore throats in this age group to 49.1% for all micro-organisms.

Influenza virus isolation dropped to 0.75% for all cases but the isolation rate for para-influenza viruses for all cases did not change much and remained at 4.8%. The isolation rate for R.S. virus in all cases increased to 7.1%, but the most surprising increase of all was that the isolation rate of herpes simplex virus increased from 0.52% to 2.2% in all cases and this may have been even higher for sore throats if separate figures were available but in figure 1 of this paper it is about 23% of the total isolates from sore throats.

Adenovirus isolation did not change much and remained/

remained at 3.4% and the rhinovirus isolation remained at 4.4%. The isolation rates 3.7% and the types of enteroviruses did not change and the isolation of coxsackie A and B viruses increased as a result of the introduction of suckling mice inoculation into the study.

Very interesting similarities with the present results are the 23% contribution of herpes simplex virus in sore throats (33.5% in the present study) and the isolation of 25.4% β. haemolytic streptococci from children with sore throats (25.8% present study).

Although separate figures are not available in Higgins et al's study the histograms for the isolation of enteroviruses, herpes simplex virus and streptococci from sore throats form a similar picture to that obtained in the present study. The virtual lack of R.S. virus in sore throats is worth noting, although these authors were inoculating the monkey kidney and Bristol HeLa cells with unfrozen specimens.

The significance of the types of enteroviruses from the sore throats does not become clear. If it had been mentioned how many of the three coxsackie A viruses isolated were from amongst the group it would have given valuable information and made the comparison more easy and clear.

Banatvala et al (1965) published the full results of their isolation from the general practice in 1965 and reported /

reported the findings of 195 cases from all age groups. Comparing the isolation of different viruses in their study with the present work, we find that they were using comparable or even better techniques with M.K. and H.Ep2 cell cultures, but no attempt was made to include a suitable system for their clinical categorisation of common cold or ARD.

Their isolation rates for influenza A virus have increased to 14.8% because of the slight epidemic between February and April 1963 in their community and they had included influenza in their study. Similarly the figure of 12.3% for the isolation of para-influenza viruses was the result of an epidemic. The lack of R.S. virus and herpes simplex virus is conspicuous. Although M type of rhinovirus could be isolated in their system their absence is not explainable.

So far as the general isolation rate of 28.2% for viruses is concerned it is understandable in the light of the above mentioned facts. Isolation rate of 7.6% for β. haemolytic streptococci is also explainable as they conducted the bacteriological examination on only the acute tonsillitis / pharyngitis cases.

Their serologically positive results of 37.9% are comparable with the 32.2% of the present study and other reports of this kind, one fact is to be remembered that the C.F.T.s in the present study were carried out by the microtechnique /

microtechnique and filter paper dried blood was used.

The value of serological investigations to supplement the virus isolation techniques is unquestionable but the method of augmenting the results by giving them in the combined form for serology and virus isolation does not justify the conclusions drawn by the authors. The figure of 62.5% success thus becomes inflated and does not take into account many overlapping factors.

The Medical Research Council report (1965) was the culmination of five years of efforts by 24 parties of workers who collaborated in a study of the aetiology of acute respiratory infections in Britain. Due to the diversity of location and personalities it has included all the respiratory tract syndromes, and the limits set for ages in the beginning were also set aside for the necessity of obtaining better figures for the completion of the study.

The controls in this study when ever investigated were selected from the same families but only one person nearest to the age of thecase was selected and called contact control.

Comparison here is only made with the sore throat cases in the M.R.C. report and the present study.

From the sore throat cases the M.R.C. party isolated influenza viruses 0.26%, para-influenza viruses 1.0%, R.S. virus 0.26%, adenovirus 8.2%, rhinoviruses 2.1%, /

2.1%, enteroviruses 8.5%, and herpes simplex virus 1.8%.

In the present study these viruses were isolated in the following percentages. Influenza B virus 3.2%, para-influenza viruses 1.9%, R.S. virus not isolated, adenoviruses 0.65%, rhinoviruses 1.6%, enteroviruses 2.2% and herpes simplex virus 4.9%.

The resemblance is remarkable in the high isolation rates of streptococci, 23.9% MR.C. and 25.8% in the present study. The higher isolation rates for viruses in the M.R.C. study may again be attributed to two factors. The isolation of coxsackie A viruses (not clear), and adenovirus which have been stated to be prevalent in certain areas of M.R.C. study. The isolation rate of 1.8% for herpes simplex virus is well within the lower limits of isolation for this virus in other studies.

The isolation rates in the M.R.C. report, the present study and two other studies amongst the controls are compared in Table XI. (Next page).

The overall isolation from controls in the M.R.C. study is very similar to the present study but the individual viruses have differed depending upon the types and frequencies of viruses isolated from the index cases. Although the figures are low they are statistically significant in both studies. (Table IB).

Higgins et al (1966) got remarkable results with the isolation for 1964. Out of 76 sore throat cases amongst the 0 - 15 year olds they isolated 35.5% viruses and /

TABLE XI

Isolation rates in percentage of viruses and β. haemolytic streptococci amongst controls in the present and three other studies.

Study	Age groups	Total Nos.	Influenza A, B and C viruses	Parainfluenza viruses	Adenoviruses	Enteroviruses	Rhinoviruses	Herpes simplex virus	All viruses	β. haemolytic streptococci	Over all viruses and streptococci
M.R.C. rep 1965	Presumably children	493	X	1.4	1.6	5.07	2.2	X	10.1	4.6	14.7
Glazen et al 1967	0 - 11	Viruses 55 Strep 164	X	X	X	1.8	X	1.8	3.6	21.9	24.2
Currie study Brown et al (unpublished)	0 - 15	104	X	X	X	5.7	X	X	5.7	4.8	10.5
Present	0 - 15	454	1.3	0.9	.66	1.3	X	2.6	6.8	7.06	13.86

and 40.7% β . haemolytic streptococci and as in the present study herpes simplex virus was the next most frequent agent after the streptococci (percentage not available) followed by enteroviruses, adenoviruses, and rhinovirus,the role of adenoviruses and rhinoviruses being reversed in the present study.

The total isolation rate for sore throat cases in 0 - 15 years for viruses and streptococci in Higgins et al's study came up to 76.2%.

The latest study directly comparable with the present study was Glazen et al's (1967) "Group A Streptococci, mycoplasmas and viruses associated with acute pharyngitis". This study was carried out in two paediatric practices in Chaple Hill, North Carolina and was continued from December 1964 onwards for one year.

Virus isolation from children with acute pharyngitis was attempted by the use of frozen specimens in Ginsberg's medium with 5% chicken serum in primary monkey kidney, HeLa and W138 foetal lung cells. This tissue culture and transport system is in general comparable and has advantage over that used in the present study. Comparison of virus and streptococcal isolation has only been made with 0 to 11 years age groups.

The virus isolation rate of 13.6% is almost similar to the 14.7% of the present study. The list of viruses isolated only differs from the present study in that no influenza /

influenza viruses were isolated, para-influenza viruses were isolated in a larger percentage of 3.2 as opposed to 1.9% of the present study. Rhinoviruses and enteroviruses were both isolated from 0.92% cases by Glazen et al while the corresponding percentage for the present study are 1.6 and 2.2% respectively. There were two marked differences from the present study, these were in the isolation rates for adenoviruses and herpes simplex virus. Glazen et al's rates for these viruses were 7.8% and 0.92% while the situation is reversed in the present study, the respective isolation rates being 0.65% adenoviruses and 4.9% herpes simplex virus.

The absence of respiratory syncytial virus in both studies might have been either due to the use of frozen specimens or the very low isolation rates for this virus from sore throat cases. (M.R.C. report 1965).

Once again the isolation rates of β. haemolytic streptococci from pharyngitis cases has proved to be of a very high magnitude as has already been seen with the previous studies. Glazen et al isolated 38.6% Group A streptococci and this has brought their total isolation up to 52.2% for viruses and streptococci combined.

Glazen et al have also used asymptomatic contacts as controls and here the isolation rates for viruses and streptococci differ considerably with the present study. In viruses they only isolated one enterovirus and one herpes simplex virus from their 55 contacts (3.6%) between

0 /

0 - 11 years of age as opposed to the 6.8% positive healthy siblings with virus in the present study and 10.1% of the M.R.C. report.

The isolation of streptococci from the controls by Glazen et al has been different from both the M.R.C. report and the present study being as high as 21.9% of the total 164 contacts in the age group range. The comparative figures for the M.R.C. and the present study are 4.6% and 7.06% respectively.

As a check on the results of the present study unpublished results were kindly supplied by Drs. Brown et al. They carried out a survey on acute respiratory illness in another general practice at Currie near Edinburgh.

The materials and methods have been the same as the present study, but instead of using healthy siblings they have used other children of the same age,without symptoms.

Fifty eight index cases from the present study fell in the same period as the Currie study, and have been compared with the cases of sore throats from that study.

From the Currie practice, five viruses were isolated 4 of which were coxsackie B_3 viruses and one para-influenza 2 virus and their isolation rate was 13.5% for viruses. During the same time two coxsackie B_3 and one coxsackie B_4 viruses were isolated from the present study while the other isolates were one para-influenza 2 virus, one adenovirus type 3 and one herpes simplex virus. The isolation rate from the 58 corresponding cases in the /

the present study was 10.3%.

Streptococcal isolation rates proved to be exactly the same being 18.9% in both studies while the overall isolation rates virus and streptococcal have been 32.0% (Brown et al) and 29.2% in the present study. Comparison with the Currie controls has been made rather enthusiastically because the two controls represented different categories of controls.

A total of 6 (5.7%) viruses were isolated from the Currie controls and all belonged to the coxsackie B viruses. Three different viruses were isolated from the 75 healthy siblings corresponding to the 58 index cases and the virus isolation rate was 4%. These three viruses isolated from the present study controls were adenovirus type 3, herpes simplex virus and poliovirus 1.

Brown et al's controls yielded 4.8% β. haemolytic streptococci.

With the examples presented and comparisons made during the discussion it became clear that the study has been completed smoothly, within the limits of its resources. The comparison of isolation rates with various studies has proved the point that a diverse variety of viruses can be isolated from the sore throat syndrome and the isolation rate may vary between 10.3% present study Currie comparison group to 35.5% (Higgins et al's final report 1966 in sore throats only).

The /

The contribution of β. haemolytic streptococci to this syndrome has long been established. By taking the averages of isolation rates from the various studies, we can say that in isolation studies from sore throats one can expect to isolate about 21.3% streptococci within a range of 13 to 35%.

The contribution of the two categories of agents (viruses and streptococci) seems to be about 50% each in sore throats.

Seasonal Isolation.

Although both viruses and β. haemolytic streptococci are indicated to be associated with the sore throat syndrome almost equally, the seasonal ratios of isolation of one group to other differed and may have a bearing on the total picture of the syndrome. (Histogram 1).

During the winter months of January to March, viruses in general seem to be more active than the rest of the year. The ratios of virus to streptococcal isolation for the quarters of January to March in 1966 and 1967 indicate a low rate of difference between the isolation of the two types of agents 22.6 and 10.86% viruses and 26.2 and 15.21% streptococci in the respective years.

As the summer months approach the isolation figures increased in favour of streptococci, being 10.7 and 6.6% for viruses and 35.3 and 27.2% for streptococci between April to June during the two summers (1966 and 1967). Between July and September 1966 the ratio of virus to streptococcal isolations evened off both resting at 30.7%, but these figures of July to September may be misleading as very few specimens were collected during this period due to organisational difficulties. There was a definite drop in the isolation of streptococci during October to December 1966, the respective figures for viruses and streptococci being 11.9 and 20.9%. Banatvala et al (1965) have noted high figures for streptococcal isolation for September to November, which went down during December, January /

January and February but again reached the peak by April.

It is very difficult to comment in a situation like this when no figures are available from previous studies to compare.

Although the figures given in Higgins et al's (1966) collective report on their studies for three years are only pertinent for the general group of acute respiratory diseases the pattern is similar except for November 1964, their general isolation rates for the streptococci have been higher during January to April building up till June.

Similar observations were made by Kendall et al (1960) but their peak was in February and kept up until March, dropped down in April and again rose in the summer months.

In viruses the isolation of Myxoviruses was particularly concentrated between October to March (Histogram 1, Hist. 3) all the influenza B virus being isolated during one month, January 1966, and all but one para-influenza virus being isolated between October 1966 and March 1967. The only exception being one para-influenza virus being isolated during August 1966. Similar observations are present in Banatvala et al's (1965) report. These findings are in agreement with the experience of Higgins et al (1966) over the winters of 1962, 1963 and 1964 as well as with Banatvala et al's (1964) and of the M.R.C. report for the same winters.

Rhinoviruses were more common between October to January in the present study; two M types were recovered during /

during January 1966 and 2 M and one H type between October and November 1967. Similar results were found by Higgins et al and the M.R.C. working party although low isolations of these viruses were observed by the above workers throughout the year.

The isolation of adenoviruses was low in the present study. They were isolated during the months of April 1966 and March 1967. Although the types of isolates differed, adenoviruses were isolated from controls during the months of May and June 1966 and February 1967. The distribution of these viruses in the index cases agrees with those observed by Higgins et al (1964), and to some extent with the M.R.C. report.

The isolation of enteroviruses was commonest between the months of March to August amongst the index cases although two viruses with enterovirus like properties were isolated from two siblings in January 1966. Once again these results are similar to those observed by Kendall et al (1960), M.R.C. report (1965) and Higgins et al (1966).

Herpes simplex virus was isolated throughout the year but the peak of isolation was between January to March 1966 when 7 out of 15 herpes simplex viruses were isolated from the index cases. Although the isolation of this virus was comparatively higher than the other viruses in controls the peaks of isolation were completely different from those in index cases being between May to June 1966 and October and November 1966. These findings are shared by Higgins et /

et al (1966) which is the only study in which similar numbers were isolated.

Age distribution.

Table IV shows the virus isolation according to the age of the patients and Table III and Hist. 2 shows the types of viruses and streptococci isolated from them.

The isolation rate of viruses only, being highest 12.5% in the 3 to 5 year age group while it was slightly lower (11.6%) in the 5 to 15 years age group. The lowest isolation rate for viruses only was in the 0 - 3 years age group (11.1%). (Table III).

The picture was completely reversed in the case of streptococcal isolations only, being the highest (55.5%) in the 0 - 3 age group decreasing to 22.0% in the 5 - 15 age group and lowest (20.8%) in the 3 to 5 years age group.

The isolation rate for myxoviruses was highest amongst the 3 to 5 year olds being 8.3%, and 4.8% of these viruses were isolated from the 5 to 15 year olds. No myxoviruses were isolated from the 0 - 3 year olds.

Rhinoviruses were isolated exclusively, 2% from the 5 to 15 year age group. Adenoviruses were isolated from 11.1% of the 9 children 0 - 3 year old and 0.4% from the 5 to 15 year olds.

Enteroviruses were exclusively isolated from 2.8% children in the 5 to 15 year age group.

Herpes simplex isolation was highest 6.2% in the 3 to /

to 5 year olds, 4.8% in the 5 to 15 year olds, and nil in 0 to 3 year old children. These findings differ from Higgins et al's (1966) observations of higher isolation rates for this virus with the increase of age.

The general age distribution of viruses is in agreement with the M.R.C. isolation and the isolations made by Higgins et al (1966). The isolation of 55.5% streptococci in 0 to 3 year olds does not agree with Higgins et al but agrees with the observations of the M.R.C. report that the streptococci were isolated more in children below 6 years than above.

Symptoms and signs. (Table X).

Observations on symptoms and signs comparable to some extent with the present study are available in Kendall et al's (1960) study, the M.R.C. report (1965) and Glazen et al's (1967) study of acute pharyngitis.

Kendall et al observed that in the three groups compared (Coxsackie B virus positive, streptococci positive and unidentified respiratory infections), sore throat, follicular exudate, lymphadenopathy and tenderness of lymph nodes were present in the highest percentage of St. pyogenes positive cases. Coxsackie B positive cases were exceeding others in only one aspect of gastro intestinal symptoms of nausea, vomiting and diarrhoea. Fever was difficult to associate with the majority of positive cases and in 84% of the unidentified respiratory infections it was present.

The symptomatology used by the M.R.C. working party (1965) /

(1965) was a bit different from the present study but the common symptoms used by the M.R.C. are given for comparison. As in other studies streptococci were most commonly associated with sore throats being associated with 80.6% of the positive cases. Streptococci were followed by Coxsackie A, adenoviruses, influenza virus, Coxsackie B viruses, rhinoviruses, Echo viruses and polioviruses. Sputum was most common with streptococci followed by the influenza virus, Echo and polio viruses, coxsackie A and B viruses, para-influenza virus, and rhinovirus. Nasal discharge was most frequently associated with Echo and polio viruses followed by rhinoviruses, para-influenza, influenza and coxsackie A viruses, streptococci and coxsackie B viruses.

Vomiting was commonest in Echo and polio viruses followed by influenza and Coxsackie B viruses, streptococci, para-influenza viruses and least with Coxsackie A viruses.

Diarrhoea was most commonly associated with the adenoviruses followed by Coxsackie B, para-influenza and influenza viruses, streptococci and rhinoviruses.

Fever was commonest in the persons from whom Echo or polio viruses were isolated, followed by the influenza viruses, adenoviruses, coxsackie A virus, streptococci, coxsackie B and para-influenza viruses.

Present study:-

Sore throats. In the present study, sore throats showed definite association with the isolation of enteroviruses, dual isolations of viruses and streptococci, Group A /

A streptococci alone, herpes simplex virus and influenza virus isolations, being present in more than 70% in all instances.

With rhinoviruses, adenoviruses (not reliable), para-influenza viruses and Group C and G streptococci the association is not certain being less than 70% in each case. A hint towards the need for further improvement of techniques is the association of 82.1% negative cases with sore throats.

Pain on swallowing:-

Was most observed in children from whom a rhinovirus or virus plus β. haemolytic streptococci of Group A were isolated followed by herpes simplex virus and children from whom no organisms were isolated.

Tonsillar exudate.

This symptom was commonest in the group with viruses plus Group C and G streptococci followed by children with enteroviruses, influenza viruses, herpes simplex viruses, streptococci alone and virus plus Group A streptococci. This was one symptom which was not observed in patients without isolations. Tonsillar exudate was the one symptom which was slightly significant statistically with $p > .05$. (Table IIB). This result explained the fact that most of the other complaints were made by the patients while this is one of the complaints which was scientifically observed by the clinicians and the results were those of the clinicians' /

clinicians' judgement.

Cervical adenopathy:-

Was most commonly observed in children with enteroviruses and with virus plus β. haemolytic streptococci belonging to Group C and G. This was followed by the children with herpes simplex and influenza virus isolations. There were more children with this complaint without isolation than there were for Group A streptococcal isolation, virus plus Group A streptococcal isolation, isolation of Group C and G streptococci, rhinoviruses, adenoviruses and para-influenza viruses.

Nasal discharge.

Nasal discharge was commonest in the presence of influenza and para-influenza viruses followed by the rhinoviruses, virus plus Group A streptococci, virus plus Group C or G streptococci and children with no isolations.

Some children with Group C and G streptococci and herpes simplex virus also had a nasal discharge.

Pyrexia:-

Was 100% in patients with enteroviruses and Group C and G streptococcal isolations followed by those with herpes simplex virus, influenza virus, Group A streptococci, virus plus streptococcal isolations. This group was followed by the children with no isolations, rhinovirus isolations and para-influenza and adenovirus isolations in the same order.

Vomiting:-

Was /

Was present in one child with para-influenza virus isolation, and in some children with no isolation at all and in children with Group A streptococcal isolation in the same order. Other children did not have any vomiting.

Diarrhoea:-

No virus or streptococci were isolated from children with diarrhoea.

Conjunctivitis.

Conjunctivitis was observed in children with Group C and G streptococcal isolation and para-influenza virus isolations followed by children with Group A streptococcal isolation and the group of children from whom no organisms were isolated.

Other complaints:-

Were commonest with rhinovirus isolations. Isolations of Group C and G streptococci, para-influenza and influenza viruses. Followed by the Group with no virus isolations, herpes simplex virus isolation. Group A streptococci, virus plus Group A streptococci and enteroviruses followed in the same order.

Diagnoses. (Table X).

Pharyngitis was the diagnosis made in the cases of adenoviruses, virus plus Group A streptococci, Group C and G streptococci, Group A streptococci and children with no isolations in the same order. This was followed by rhinovirus isolations, herpes simplex virus isolations, influenza and para-influenza virus isolations, enterovirus isolations /

isolations and virus plus Group C and G streptococcal isolations serially.

Follicular Tonsillitis.

Was most commonly diagnosed in children with a virus plus Group C and G streptococcus, enterovirus, herpes simplex virus, influenza virus, rhinovirus, para-influenza and Group A streptococcal isolation. Followed by the children with no virus isolation, Group C and G streptococcal isolations and virus plus Group A streptococcal isolations.

Other diagnoses:-

Were made in para-influenza virus isolations, influenza virus isolations, in children with no virus isolations, and Group A streptococcal isolation in the same order.

From the above description the dominant patterns in the present study are found for the association of the following viruses and streptococcal Groups.

Enteroviruses - in sore throat, cervical adenopathy, pyrexia, pain on swallowing.

Rhinoviruses - pain on swallowing, sore throats, cervical adenopathy, pyrexia and nasal discharge.

Herpes Simplex Virus - pain on swallowing, pyrexia, cervical adenopathy, sore throat and tonsillar exudate.

Influenza B virus - cervical adenopathy, pyrexia, sore throat, pain on swallowing, nasal discharge, tonsillar exudate and some other complaints.

Para-influenza viruses - sore throats, pain on swallowing, pyrexia, cervical adenopathy, nasal discharge, conjunctivitis, vomiting and other complaints.

Adenovirus /

Adenovirus - sore throat, pain on swallowing, pyrexia, cervical adenopathy.

Virus plus Group A streptococci - sore throat, pain on swallowing, cervical adenopathy, pyrexia, nasal discharge, tonsillar exudate.

Virus plus Group C and G streptococci - sore throat, cervical adenopathy, tonsillar exudate, pyrexia, nasal discharge and pain on swallowing.

Group C and G streptococci - pyrexia, cervical adenopathy, sore throats, pain on swallowing and other complaints.

Group A streptococci - sore throats, pain on swallowing, cervical adenopathy, pyrexia, tonsillar exudate.

As observed by Glazen et al (1967) in their study of acute pharyngitis the symptoms can not be depended upon as they are not well marked and do not appear to make distinct clinical syndromes. Glazen et al also got more children with no isolation representing each finding than the positive cases. One finding in their study of exudate being associated with Group A streptococci is missing in the present study.

However, according to the present reciprocal analysis enteroviruses, Group A streptococci, herpes simplex virus, myxoviruses, rhinoviruses and adenoviruses (results not to be depended as represent only 50% of the isolates) and the dual virus and streptococcal isolations seem to be associated with the severity of symptoms in the syndrome of sore throats as represented by pain on swallowing, cervical adenopathy and pyrexia in children in the same order. This is supported by Higgin's (1966b) description of individual virus and streptococcal association with sore throats.

Statistical Analysis.

The relationship between positive laboratory findings and clinical symptoms and diagnoses have been tested for statistical significance by χ^2 for groups (i) virological positive only (VO) (ii) streptococci positive only (SO), (iii) positive for both virus and streptococci (B), (iv) negative for both viruses and streptococci (N).

With these 4 groups and 3 degrees of freedom, a χ^2 of 3.84 would not be significant with a probability of less than 0.05 but a comparison between two specific groups may be significant at this level.

For this reason the "partitioning of χ^2 method" as described by Maxwell (1961) has been used.

The results are given in Table IIB. These results indicate that there is no statistically significant difference in the relative frequency of sore throats among patients with and without positive laboratory findings; however there is a slight significance that when both virus and streptococci are isolated the symptom, sore throat is more commonly present.

Another point of borderline significance is the presence of tonsillar exudate in the whole combination, but this condition is significant when the groups of virus positive (VO) and streptococci positive (SO) and virus positive streptococci positive (B) are compared with the negatives for both (N) thus signifying that in presence of a virus or virus plus streptococci this symptom is of importance. /

importance.

In other cases comparison is made for the significance of various isolation results between the index cases and healthy siblings by the use of direct Chi squared test in 2 x 2 tables. (Downie and Heath 1965).

These results and their significances are given in (Table IB). The results indicate that with the numbers in question and 1 degree of freedom only the comparisons between total virus positive index cases versus total virus positive controls and total streptococcal positive index cases versus total streptococcal positive siblings are significant.

Among the individual viruses the numbers being very small only the isolation of rhinoviruses among the index cases and their absence in siblings was significant.

Serological experiments.

Serological investigations have been a handy tool for confirming respiratory virus infection since the early days of influenza A virus. After the discovery of adenoviruses they have been used to augment the results of virus isolation or simply to demonstrate experiences with specific viruses by many workers.

Stanley et al (1953) supported their isolation of the new hepato-encephalomyelitis virus by showing the presence of antibodies after "infection". Parrott et al (1954) described the outbreak of febrile pharyngitis and supported their argument for infection by the increase of C.F. and neutralizing antibodies.

Jensen et al's (1955) studies of the serologic evidence of American experience with new born pneumonitis virus (type Sendai) led to the chain of controversial identifications of para-influenza 1 virus with the new born pneumonitis virus.

Other workers since then have been presenting valuable information regarding the epidemiology and possible role of various viruses in respiratory infections. Hilleman et al (1955a) in the epidemiology of RI-67 Group (Adenoviruses), Cockburn et al (1956) the retrospective confirmation of an APC3 virus (Adenovirus) infection in Colorado in 1951, Van der Veen and Kok (1957) produced 31.7% serological evidence of infection by the adenoviruses. Van Horne et al (1957) demonstrated the persistence of neutralizing titres to adenovirus and Jordan (1957) reported on the frequency of

infection /

infection with adenovirus in a family study population with the help of neutralizing antibodies. Sommerville (1957) presented the evidence of new born pneumonitis infection in South West Scotland with the help of serology.

Wenner et al (1957) in their observations on Adenovirus infection in Kansas families found 2.6% isolation rate for adenovirus 2 and 3 from 153 individuals but obtained a 20% sero conversion, thus laying down the foundations for the utility of sero conversions. Chanock et al (1959) presented the evidence that the virus isolation results of 6.2% for haemadsorption virus in 1738 children*.

This description of various studies was presented to emphasize the need for a serological investigation in addition to virus isolation. Serological results are more frequently positive than the isolation results and the techniques in the near future may not provide the same rates and figures, for these two sides of the virological work, but concurrent serological investigations provide a valuable check ~~the~~ and support for the isolation results.

In the series of studies which are being discussed as the comparable studies in general practice, serological investigations are usually incomplete or have been impossible to conduct. In only one instance was there presented (Banatvala et al 1965) serological confirmation of virus infection in 37% cases as opposed to 28.2% virus /

* rose to 19.5% when serological evidence was considered.

virus isolation from all cases. However the serological diagnosis was made only in adults. Higgins et al only mentioned serology in 9 cases in their first report of (1963) when all cases were negative and advocated more use of serology in their (1966) report.

Serology in the present study was only performed in 34 cases but in these 34 cases, 11, (32.4%) children showed a rise in titre to viruses (Table VII). This investigation being conducted by the microtechnique, the rule of ~~obtained~~ four fold rises in titres had to be relaxed in one case because of the initial dilution of material to 1:16; and isolation of virus from the throat specimens. As mentioned in the results these titres may represent one step or two fold higher titres on the macrotechnique scale. Thus a 1:16 titre may be 1:32 and so on, but the titres below 1:16 will still have to be considered as zero. A very important fact is the rise in titre of from 0 to 1:16 in the only one case from which a virus was isolated.

Other studies in which serology was conducted and which confirm the present results of higher serological determination rates are Parrott et al (1963) when they obtained 19% serologically positive results in para-influenza virus infection against 6% positive isolations, Robinson et al (1962) obtained 26% isolation and 34% serologically positive results.

Urquhart et al's (1965) results indicate that virus isolations alone were made in 6% of this study, while the category of virus infection which included the patients with /

with a four fold rise in titre with or without virus isolation contained 22% patients. This indicates the importance of virus isolations with serology, as it makes the simple virus isolation without serology a doubtful proposal for consideration as a proof for viral aetiology.

In the light of the above discussions it is concluded that the demonstration of serological evidence of viral infection is essential for any investigation of the present nature. It has two advantages over simple isolation of viruses and their comparison with a group of control population. The first advantage is that it gives a definite proof of infection in the persons concerned by the virtue of a rise in the antibody titre during the time the patient was clinically ill. The second advantage is in surveys of the present nature, by taking the blood specimens and proving the rise in titre in presence of clinical illness and virus isolation the results can be relied upon more confidently. Performing the isolation studies on a group of control population always raises the points of ratios of apparent to inapparent infections as well as the difficulties of selecting the two groups to match in all aspects of socio economic and ecological factors.

Filter paper dried blood and the microcomplement fixation technique.

The use of filter paper discs or strips by other workers in various medical and veterinary studies has been mentioned in the introduction.

Filter paper dried blood has been used in complement fixation tests for adenoviruses, R.S. virus, influenza virus/

virus and para-influenza III viruses by Brody et al (1964). Their results as in the present study showed one dilution lower titre in dried blood than the use of serum. One fact worth mentioning about the results is that (with one exception) whenever antibody titres were observed they were against viruses currently prevalent at the time.

The results of the present study and that carried out by Brody and others indicate the feasability of this method being used more commonly in virological studies than at present, the greatest asset of the method being that it is less traumatic to the patient. In studies like the present and in seroepidemiological studies the person (or parents of the child) giving the blood are not readily prepared to allow vein puncture. This fact simply reflects the human nature that unless the individual thinks the procedure will be inevitable for his benefit he is not ready to accept the pain.

On the other hand properly explained, people do not object to a small pin prick to help the cause of science. The method contains the valuable advantage of being performed with great saving of materials difficult to prepare and needless to be wasted. Given a proper chance the method is bound to catch up with the macro-complement fixation tests.

Evaluation of the efficiency of the filter paper method and the microtechnique.

In the absence of human sera and blood specimens with high titres it became necessary to immunise rabbits to confirm the results obtained by the filter paper method and the microtechnique. The diversity of the response in the /

the three pairs of rabbits immunised by different procedures makes it pertinent to bring this aspect of the study into the discussion.

As observed by many other workers the rabbits produced different responses to different antigens, routes and schedules of immunisation. Determination of the particular group of antibody molecules became evident when the nature of the loss of titre in the present method came under discussion.

The rabbits were immunised as described in laboratory methods.

To determine the particular class of immunoglobulin 19S or 7S that might be causing the loss of titre in the filter paper technique sera were treated with mercaptoethanol which affects the activity of 19S antibody. The results showed the difference of behaviour in the complement fixation tests of mercaptoethanol treated sera from the three groups of rabbits indicating the presence of different types of antibody.

The difference in behaviour is obvious as shown in the results and can be explained by the experience of other workers. (Table VIII).

In the first pair of rabbits as indicated by the results on rabbit 1 the titres produced by immunization were reduced greatly by the mercaptoethanol treatment indicating the presence of 19S antibody.

This response co-incides with the observations of Bauer and Stavitsky (1961) in their experience with rabbits primed with Salmonella before the injections of diphtheria toxoid /

toxoid and of Svehag and Mandel's (1962) observations with lower doses of polio virus in rabbits. Uhr and Finkelstein (1963) also noticed this sort of response in guinea pigs, where some animals formed precipitating 19S antibody without detectable 7S antibody after the first injection and formed 19S antibodies in response to secondary injections.

Whether we take the response of these rabbits as one in which they were given lower doses (in comparison to the other groups) or a response to priming with antigen and adjuvant at the 1st injection or the third type, observed by Uhr and Finkelstein (1963) the results are understandable.

The results of the second pair represented by rabbit 4 are more complicated as they represent a response with mixed 7S and 19S antibodies to a single intravenous dose of antigen.

The initial titres were rather high for both antigens in the three samples, but they dropped on the 3rd bleeding on the 10th day to: blood, influenza A 1/16, B 1/16, serum, influenza A 1/32 B 1/32 and mercaptoethanol treated serum 1/32 1/32 and were found to be increased to the levels shown in the results on the 88th day.

In the immune samples we find that the results for one antigen are not affected by the treatment with mercaptoethanol indicating the presence of mixed 19S and 7S content for this bleeding.

This type of response of the initial production of 19S /

19S being replaced by 7S antibody has been observed by many workers. Such a response was observed by Mathies and Stavitsky (1962) in immunisation of rabbits against T_2 bacteriophage by Svehag and Mandel (1962) in the response to the high dose of poliovirus in rabbits and in the response of guinea pigs to a single injection of ØX174 by Uhr and Finkelstein (1963).

The response of the 3rd pair of rabbits as represented by results on rabbit 5, is a simple example of an infection with a virus.

In these rabbits there was very little antibody to the virus present before infection which reached a high peak after the immunisation and antibodies being 7S at this stage were not effected by the mercaptoethanol treatment. There is agreement amongst the workers on this type of response to an infection. There is early production of 19S antibody which is slowly replaced by more persisting 7S antibody.

Cowan and Trautman (1965) observed this in guinea pigs by infection with foot and mouth disease virus. Lehrich et al's (1966) finding on adenoviral infections of human volunteers and Waldman et al's (1967) findings with influenza A2/Bethesda infection of human volunteers also include similar observations.

Immunoelectrophoresis experiment:-

This experiment was carried out to find out if any qualitative correlation between the complement fixation titres and the precipitates can be obtained. The results indicate /

indicate that there is such a possibility.

Although the experiments in the present study were carried out with the hope that the specific loss of titres due to 19S antibody would be easier to confirm by this method this aim could not be achieved.

The reason for the limited success of the experiments lies in the fact that antisera to the specific fractions (19S, 7S etc.) were not available. The only conclusions that could be drawn were that a qualitative increase or decrease in the samples titres as shown by the complement fixation tests could be confirmed. The success of the tests was limited also in the other respect that the rises could be compared properly only in individual rabbits. The quantity of the precipitates although proportional in individual rabbit samples to the CFT titres differed variously in the three rabbits whose results are presented in the photographs (Figs. 1A, 1B, 2A, 2B, 3A and 3B).

The results of microcomplement fixation tests using filter paper dried blood from children and immune rabbits have proved this technique to be successful.

The comparison of results from dried blood, serum and mercaptoethanol treated serum has removed the doubts about the loss of activity due to any specific antibody fraction.

However the minor loss of activity in the filter paper dried blood is to be accepted and has been observed by other workers also Brody et al (1964) and qualitative immunoelectrophoresis /

immunoelectrophoresis in the present study has confirmed the above experiences.

The results as represented by the complement fixation tests on children's sera have proved the point that the technique can be used successfully and comparable results to macrocomplement fixation tests can be achieved with all the inherent advantages of the microtechnique.

PART V.

Summary

1. The present study describes the virological investigations carried out on children between infancy and 15 years of age suffering from sore throats or similar complaints and diagnosed as pharyngitis or follicular tonsillitis. These patients were all in the same general practice.

2. Specimens from 306 index cases were examined for the presence of viruses. 45 viruses, (14.7 per cent) were isolated from these children. Herpes simplex virus was the commonest and was isolated from 15 index cases, (4.9 per cent), followed by influenza B virus 10, (3.26 per cent), enteroviruses 7, (2.2 per cent), para-influenza virus 6, (1.9 per cent), rhinoviruses 5, (1.6 per cent), and adenoviruses from 2 (0.65 per cent).

   The isolation rate of haemolytic streptococci from the index cases was (25.8 per cent).

3. The highest percentage of viruses was isolated from 3 to 5 year olds being (16.7 per cent) followed by (14.4 per cent) in 5 to 15 year olds and (11.1 per cent) in 0 to 3 year olds. Highest percentage of streptococci was isolated from 0 to 3 year olds being (55.5 per cent).

4. Seasonal isolation results indicated a general dominance of myxoviruses and rhinoviruses from autumn /

autumn and winter and enteroviruses and adenoviruses from spring till summer. Herpes simplex virus was isolated throughout the year. The isolation rate of viruses was significantly higher in the winter than in the summer. The reverse of this was true for haemolytic streptococci.

5. The association of various symptoms with viruses and streptococci was ascertained and significance evaluated.

6. Specimens from 753 healthy persons including 454 healthy siblings of the same age groups and 299 adults and 15 plus teenage members of the family were also examined for virus isolation.
Viruses were isolated from 6.8 per cent of the healthy siblings and β. haemolytic streptococci from (7.06 per cent) in this group. The differences in the isolation rates among the index cases and healthy siblings have proved to be statistically significant. Of the adult and juvenile members of the family (3.01 per cent) were positive for viruses and (2.3 per cent) for streptococci.

7. Studies in 247 families indicated that multiple episodes were more likely to occur in the medium (7 to 9 members) and large (10 or more members) families.

8. Before carrying out serological tests on the children's sera the use of micro- and macro- complement fixation tests /

tests was compared. It was found out that there were slightly lower titres with the use of filter paper dried blood from finger pricks.

9. Micro-complement fixation tests were then carried out on acute and convalescent specimens of filter paper dried blood from 34 children. Ten children showed a 4 fold or more rise to one or more respiratory virus antigens and one child from whom a herpes simplex virus was isolated showed an initial rise from zero to 1:16 against the herpes simplex antigen. Altogether (32.3 per cent) specimens were serologically positive as opposed to the (14.7 per cent) of the throat swabs that were positive for virus isolation.

10. On the observation of general low titres in the specimens from the index cases using filter paper dried blood with micro-techniques, it was decided to test the method more thoroughly.

    Three pairs of rabbits were immunized by three different schedules. The results of immunized rabbit dried blood using the micro-technique were compared with the result of serum by the micro- and macro-techniques. Usually one step lower titres were obtained with the blood eluates than the serum samples with the use of micro-technique.

11. The suggestion that 19S globulin fraction may be responsible /

responsible for loss of titres in the filter paper dried blood was investigated. A comparison of the titres of mercaptoethanol treated serum with the titres of dried blood eluates and whole serum was made. No correlation was obtained between the treatment of serum with mercaptoethanol and the loss of titres by the filter paper method. It was concluded that the loss of titre in the filter paper method is not due to specific adsorption of 19S globulin but was due to the repeated handling of small portions of blood.

12. Qualitative correlation of complement-fixation titres of the mercaptoethanol treated serum, whole serum and filter paper eluates of dried blood was obtained by the use of immunoelectrophoresis.

13. The significance of the present results has been discussed in the light of the observations of other workers who have presented comparable data.

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A P P E N D I X.

APPENDIX.

TABLES, HISTOGRAMS AND FIGURES.

TABLES.

X. /

VIRUS TABLES.

HISTOGRAMS.

FIGURES.

Page.

Photographs of Lines of precipitates after immunoelectrophoresis of filter paper eluates, dialyzed serum and mercaptoethanol treated serum. (Developed against goat anti serum to rabbit serum).

TABLES.

TABLE IA

Number and Percentage of Viruses and β. haemolytic Streptococci isolated from Index-Cases.

CONTROLS AND ADULT CONTACTS

ORGANISMS	Index-Cases 0 - 15 years		Controls 0 - 15 years		Contacts 15+ and adults	
	Number of isolations	% of total investigated	Number of isolations	% of total investigated	Number	% of total investigated
Viruses	45	14.7	31*	6.8	9	3.01
β. haemolytic Streptococci	79	25.8	32	7.06	7	2.3
Viruses + β. haemolytic Streptococci	(9)+	(2.94)+	(6)+	(1.3)+	0	0
Index cases or controls with no viruses or β. haemolytic Streptococci	182	59.8	391	85.9	283	94.0
Total patients	306"	100	454	100	299	100

* includes 30 controls
\+ included in viruses and streptococci having no separate significance
" corresponds to number of persons examined.

TABLE IB.

Relationship between the Virologically and Bacteriologically Positive Index-Cases and healthy siblings (clinically positive Index-Cases and clinically negative siblings). Statistical significance if any.

Description	χ^2	Degree of freedom.	Probabilities	Conclusions
Total virus positive Index-Cases and total virus positive siblings	11.74	1	p.>.001	Significant
Total Streptoococci positive Index-Cases and siblings	50.134	1	p >.001	Significant
Influenza B virus in Index-Cases and siblings	2.482	1	p <.2	Non significant
Para-influenza viruses in Index-Cases and siblings	0.915	1	p <.5	Non significant
Rhinovirus in Index-Cases and siblings	5.176	1	.02<p <.05	Significant
Adenovirus in Index-Cases and siblings	0.220	1	p <.7	Non significant
Enteroviruses in Index-Cases and siblings	0.521	1	p <.5	Non significant
Herpes simplex virus in Index-Cases and siblings	2.103	1	p <.2	Non significant

TABLE IIA

Numbers of Viruses and β. haemolytic streptococci isolated from Index cases and their representing symptoms and diagnosis.

	PRESENTING SYMPTOMS											DIAGNOSIS		
Organisms isolated	Totals	Sore throat	Pain on swallowing	Tonsillar exudate	Cervical adenopathy	Nasal discharge	Pyrexia	Vomiting	Diarrhoea	Conjuncti-vitis	Additional complaints	Pharyngitis	Follicular tonsillitis	Other diagnosis
Viruses only	36	26	28	10	29	10	25	1	0	1	12	18	14	4 Echo ? Laryngitis Scarlet fever U.R.T.I.
Virus + β.H.S. Group A	6	6	6	1	5	2	4	0	0	0	1	5	1	0
Virus +β.H.S. Group C	3	3	1	2	3	1	2	0	0	0	0	1	2	0
β.H.S. Group A only	65	56	55	17	55	12	45	5	0	1	14	41	19	5-sore throat 2 Scarlet fever 2 Sinusitis 1
β.H.S. Group C or G only	5	3	2	1	4	1	5	0	0	1	2	4	1	0
No organisms isolated	191	157	167	29	170	49	118	16	7	3	56	120	57	14
GRAND TOTALS	306	251	259	60	266	75	199	22	7	6	85	189	94	23

β.H.S. = β. haemolytic streptococci

The assessment of the relationship between laboratory findings and clinical diagnoses as tested for statistical significance by χ^2.

Four groups were studied, Virological positive only, (VO), Streptococcal positive only (SO), Positive for both viruses and streptococci (B), and negative for virology and bacteriology (N).

TABLE IIB

Relationship between symptoms, diagnosis and Laboratory findings.

(a) Overall relationship. d.f = 3
(b) Specific comparisons which are statistically significant if any, over all comparison. d.f = 1

Signs symptoms and laboratory findings	χ^2	Degrees of freedom	Probabilities	Conclusions
Sore Throat	4.565	3	.2 <p <.3	Not significant
Virologically Positive only compared with Streptococcal positive and (Viral + Streptococcal positive)	3.768	1	.05 <p <.1	Questionable significance
Pain on swallowing	3.332	3	.3 <p <.4	Not significant
Tonsillar exudate	6.628	3	.05 <p <.1	Questionable significance
Viral positive or Streptococci positive				
Viral positive Streptococci negative)	6.312	1	.01 <p <.025	Significant
Cervical adenopathy	2.472	3	.4 <p <.5	Not significant
Nasal discharge	2.056	3	.5 <p <.6	Not significant
Pyrexia	2.442	3	.4 <p <.5	Not significant
Vomiting	2.069	3	.5 <p <.6	Not significant
Diarrhoea	NOT RELEVANT			
Conjunctivitis	0.4598	3	.8 <p <.9	Not significant
Additional symptoms	0.333	3	.7 <p <.8	Not significant
Pharyngitis	2.4810	3	.4 <p <.5	Not significant
Follicular tonsillitis	1.378219	3	.7 <p <.8	Not significant
Other diagnoses	.1455	3	.97 <.99	Not significant

TABLE III

Number and Percentage of Isolation of Viruses only, β. haemolytic streptococci only and Viruses + β. haemolytic streptococci from Index cases and Controls in various age groups.

AGE Group		Total	Virus only		β. haemolytic streptococci only.		Virus + β. haemolytic streptococci		No isolation	
			No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage.
0 - 3	Index	9	1	11.1	5	55.5	0	0	3	33.3
	Controls	129	12	9.3	6	4.6	2	1.5	109	84.4
3.1/12-5	Index	48	6	12.5	10	20.8	2	4.2	30	62.2
	Controls	80	4	5.0	7	8.7	0	0	69	86.2
5.1/12-15	Index	249	29	11.6	55	22.0	7	2.8	158	63.4
	Controls	245	9*	3.6	13	5.3	4*	1.6	217	88.9

One child of 8, 2681 had two Viruses + streptococci

Table IV

Percent Virus isolations by Age
in Index Cases and Controls

AGE	No. of Index Cases	With Virus Isolation		No. of Controls	With Virus Isolation	
		No.	%		No.	%
0 - 3	9	1	11.1	129.	14	10.8
3.1/12 - 5	48	8	16.6	80	4	5.0
5.1/12 - 15	249	36	14.4	245*	13*	5.3*
Total	306	45	14.7	454	31	6.8

* One child yielded two viruses.

TABLE IV.

Percent virus isolations by age in index cases and controls.

TABLE V

Distribution of Viruses in Index-Cases and Controls

VIRUSES	INDEX CASES					CONTROLS				
	Total	%	0 - 3	3.1/12-5	5.1/12-15	Total	%	0 - 3	3.1/12-5	5.1/12-15
Influenza B virus	10	22.2	-	2	8	6	19.3	2	-	4*
Para influenza 1	4	13.3	-	2	2	2	12.9	2	-	-
Para influenza 2	2		-	-	2	2		-	1	1
Rhinovirus M	4	11.1	-	-	4	-	0	-	-	-
Rhinovirus H	1		-	-	1	-		-	-	-
Adenovirus 1	1	4.4	1	-	-	-	3.2	-	-	-
Adenovirus 3	1		-	-	1	1		1	-	-
Adenovirus 5	-		-	-	-	2	6.4	1	-	1
Poliovirus 1	-		-	-	-	1	6.4	1	-	-
Poliovirus 3	-	-	-	-	-	1		1	-	-
Echo 27	1	15.4	-	-	1	-	12.9	-	-	-
Coxsackie B_3	2		-	-	2	-		-	-	-
Coxsackie B_4	1		-	-	1	-		-	-	-
Enterolike unidentified	3		-	-	3	2		2	-	-
Enterolike lost	-		-	-	-	2		2	-	-
Herpes Simplex Virus	15	33.3	-	3	12	12	38.7	3	3	6*
	45	100	1	7	37	31	100	15	4	12*

* One Control 26 B_1 yielded two viruses.

TABLE VI.

Results of the Micro-complement fixation tests on filter paper eluates of blood from Index-Cases.

- = <16

INDEX NO.	Sera	Influenza A	Influenza B	Influenza C	Sendai	Adenovirus	Respiratory Syncytial Virus	Herpes Simplex
I235	Acute	-	-	-	-	-	-	-
	Conv	-	-	-	32	16	-	-
I239	Acute	16	16	-	-	-	-	-
	Conv	16	16	-	-	-	-	16
I244	Acute	-	-	-	-	-	-	-
	Conv	-	-	16	32	16	32	32
I259	Acute	-	-	-	-	-	-	16
	Conv	-	16	16	-	-	-	64
I260	Acute	-	-	-	-	-	-	-
	Conv	-	-	32	-	-	-	-
I261	Acute	-	-	-	16	-	-	-
	Conv	-	-	16	16	32	-	-
I263	Acute	-	-	-	-	-	-	-
	Conv	-	-	-	-	-	-	128
I268	Acute	16	-	-	16	-	-	-
	Conv	16	-	-	16	32	-	-
I271	Acute	-	-	-	-	-	-	-
	Conv	-	-	32	-	16	-	-
I291	Acute	-	-	-	-	-	-	-
	Conv	-	64	-	-	16	-	-
I295	Acute	16	-	16	-	-	-	32
	Conv	16	16	16	16	32	-	16

TABLE VI.

Results of the micro-complement fixation tests on filter paper eluates of blood from index cases.

TABLE VII

Correlation of clinical symptoms, diagnoses, serology and virus isolation in serologically positive cases.

Index No.	Sore throat	Pain on Swallowing	Tonsillar exudate	Cervical adenopathy	Nasal discharge	Pyrexia	Vomiting	Diarrhoea	Conjunctivitis	Others	Pharyngitis	Follicular tonsillitis	Increase in titres for	Virus isolation
I 235	+	+	-	+	-	+	-	-	-	Abdominal pain	-	+	Sendai. Adeno.	X
I 239	+	+	-	+	-	-	-	-	-	-	-	+	Herpes	Herpes simplex
I 244	+	+	-	+	-	-	-	-	-	Catarrh	+	-	Flu C, Sendai, R S, Herp.	X
I 259	+	+	-	+	-	-	-	-	-	-	+	-	Flu B, C Herpes	X
I 260	+	+	+	+	-	+	-	-	-	-	-	+	Flu C	X
I 261	+	+	-	+	-	+	-	-	-	-	+	-	Flu C Adeno	X
I 263	+	+	-	-	-	+	-	-	-	-	+	-	Herpes	X
I 268	+	+	-	+	-	+	-	-	-	-	+	-	Adeno	X
I 271	+	+	-	+	-	+	-	-	-	-	+	-	Flu C, Adeno	X
I 291	+	+	-	-	-	+	-	-	-	Backache	+	-	Flu B, Adeno	X
I 295	+	-	+	-	-	+	-	-	-	-	+	-	Flu B, Sendai Adeno	X
TOTAL 11	11	10	2	8	0	8	-	-	-	3	8	3	X	1

TABLE VIII.

Results of CFTs. with filter paper dried blood, serum and mercaptoethanol treated serum.

Results of C.F.Ts . with filter paper dried blood, Serum and Mercaptoethanol treated serum.

Rabbits	Blood eluate		Serum		M.E. Treated serum	
1st Pair Influenza Vaccine + Adjuvant intradermally 0.5ml. day Zero 0.2ml. day 80th	Flu A	Flu B	Flu A	Flu B	Flu A	Flu B
Rabbit 1						
Preimmunization	0	0	32	16	16	16
Day 92nd after immunization	128	64	512	128	16	32
Rabbit 2						
Preimmunization	32	0	32	16	16	16
Day 92nd after immunization	128	32	256	128	16	16
2nd Pair Single dose of 0.5ml. Influenza vaccine intravenously day 0	Flu A	Flu B	Flu A	Flu B	Flu A	Flu B
Rabbit 3						
Preimmunization	0	0	0	0	0	0
92nd day after immunization	128	256	512	256	64	64
Rabbit 4						
Preimmunization	64	64	128	64	128	64
88th day after immunization	128	128	128	64	64	16
3rd Pair Infection with herpes simplex virus on day 0	herpes simplex		herpes simplex		herpes simplex	
Rabbit 5						
Preimmunization	16		32		16	
92nd day after immunization	128		512		256	
Rabbit 6						
Preimmunization	64		128		64	
92nd day after immunization	256		512		256	

TABLE IX

Results of the initial complement fixation tests on six rabbits using filter paper dried blood and serum by Micro and Macrotechniques.

Rabbit No.	Filter paper dried blood Microtechnique.		Serum Microtechnique		Serum Macrotechnique	
	Influenza* A	Influenza * B	Influenza A	Influenza B	Influenza A	Influenza B
1 92 day	128	64	512	128	512	128
2 92 day	128	32	256	128	128	128
3 92 day	128	256	512	256	256	256
4 88 day	128	128	128	64	128	128

Rabbit No.	Herpes Simplex *	Herpes Simplex	Herpes Simplex
5 92 day	128	512	512
6 92 day	256	512	512

* Antigens used.

TABLE X

Association of symptoms and diagnosis with virus and streptococcal association.

Percentages of symptoms and diagnosis with various isolation combinations.

Virus	Sore throat	Pain on swallowing	Tonsillar exudate	Cervical adenopathy	Nasal discharge	Pyrexia	Vomiting	Diarrhoea	Conjuncti-vitis	Other complaint	Pharyngitis	Follicular tonsillitis	Other diagnosis
Influenza B virus	70	50	40	90	50	70	X	X	X	30	50	40	10
Parainfluenza virus	66.6	66.6	X	50	50	50	16.6	X	16.6	33.3	50	33.3	16.6
Rhinoviruses	60	100	X	60	40	60	X	X	X	60	60	40	X
Adenoviruses	50	50	X	50	X	50	X	X	X	X	100	X	X
Enteroviruses	100	85.7	57.6	100	X	100	X	X	X	14.2	42.8	57.1	X
Herpes Simplex virus	80	93.3	33.3	93.3	20	75	X	X	X	26.6	53.3	46.6	X
Virus + H.S. Gp. A	100	100	16.6	83.3	33.3	66.6	X	X	X	16.6	83.3	16.6	X
Virus + H.S. Gp. C or G	100	33.3	66.6	100	33.3	66.6	X	X	X	X	33.3	66.6	X
β. H.S. Gp. A	86.1.	84.6	26.1	84.6	18.4	69.2	7.3	X	1.5	21.5	63	29.2	7.2
β. H.S. Gp. C or G	60.0	40.0	20.0	80.0	20.0	100	X	X	20	40	80	20	X
No isolations	82.1	87.7	15.1	89.0	25.6	61.7	8.3	3.6	1.5	28.7	62.7	29.3	7.3

FAMILY ISOLATION TABLE.

VIRUSES FROM CONTACTS ACCORDING TO SEASON, TYPE AND ASSOCIATION.

SIBLINGS

	CODE Nº	RELATION	AGE YEARS	SEX	Index same virus + ve	Index other virus + ve	Index virus - ve	FAMILY SIZE	SEASON
	294	S3	4	F	x	x	√	7	□
	210	S2	5	F	x	x	√	7	○
	203	S1	14	F	x	x	√	7	○
	171	B1	5	M	x	x	√	5	○
	147	S	11	F	x	x	√	8	●
	168	S1	7	F	x	x	√	6	○
	113	S3	9	F	√	x	x	9	●
	113	B1	9	M	√	x	x	9	●
	113	S1	13	F	√	x	x	9	●
↑	26	B1	8	M	x	x	√	7	△
↑	8	S3	1	F	x	x	√	11	△
Herpes Simplex Virus	7	S1	2	F	x	√	x	5	△
↑	28	S	3	F	x	√	x	5	△
Entero like Lost	28	B	1	M	x	√	x	5	△
↑	178	B3	2	M	x	√	x	9	○
Entero like Unidentified	178	B2	3	M	x	√	x	9	○
Polio Virus 3	128	B4	3	M	x	x	√	5	●
Polio Virus 1	273	S	9mth	F	x	x	√	4	■
↑	139	B1	1	M	x	√	x	5	●
Adenovirus 5	122	S2	6	F	x	√	x	5	●
Adenovirus 3	252	B1	9	M	x	x	√	5	■
	181	B4	4	M	√	x	x	8	○
Para influenza 2 Virus	181	B1	15	M	√	x	x	8	○
	195	S3	1	F	x	x	√	8	○
Para influenza 1 Virus	168	S2	3	F	x	x	√	6	○
	27	B1	10	M	√	x	x	8	△
	26	S2	1⅓	F	x	x	√	7	△
	26	B2	7	M	x	x	√	7	△
	26	B1	8	M	x	x	√	7	△
↑	22	S	8	F	√	x	x	6	△
Influenza B Virus	17	B	3	M	√	x	x	7	△

△ JAN - MAR '66
● APR - JUNE '66
JULY - SEPT '66
○ OCT - DEC '66
■ JAN - MAR '67
□ APR - JUNE 67

✓ = Present

X = Absent

ADULTS OR 15+

	CODE Nº	RELATION	RELATIONSHIP	Index same virus + ve	Index other virus + ve	Index virus - ve	Virus in other family members	FAMILY SIZE	SEASON
	255	M	Moth	x	x	√	x	5	■
	208	F	Far	x	x	√	x	6	○
	138	M	Moth	x	x	√	x	7	●
↑	135	M	Moth	x	ENT U.I.	x	x	6	●
Herpes Simplex Virus	71	F	Far	x	x	√	x	5	△
Entero like Unidentified	23	S1	Sis 17	x	x	√	x	5	△
	29	M	Moth	√	x	x	x	4	△
↑	18	M	Moth	x	x	√	x	6	△
Influenza B Virus	6	M	Moth	x	RHINO M	x	x	4	△

TABLE XIII.

Comparison of families with single or multiple episodes of sore throat.

Episodes	Total families	Total Index Cases	Index cases Virus Positive		Index cases β. H.S. Positive		Family size Normal		Medium		Large	
			No.	%	No.	%	4-6 Persons No.	% of total	7 to 9 Persons No.	% of total	10< persons No.	% of total
Single	175	175	22 *	12.5	55 +	31.4	136	77.7	34	19.4	5	2.8
Multiple	47	106	18 *	16.9	22 +	20.7	31	65.9	14	29.7	2	4.2
Not done	25	25	5	20	2	8	x	x	x	x	x	x

* Statistically significant difference χ^2 = 7.202 1df .01<p <.001

\+ Statistically significant difference χ^2 = 7.106 1df .01<p <.001

VIRUS TABLES.

VIRUS TABLE I.

Herpes Simplex Virus from Index-Cases.

Index No.	Age and Sex	Mo	Sore Throat	N	Pain on swallowing	O	Tonsillar exudate	P	Cervical adenopathy	Q	Nasal discharge	R	Pyrexia	S	Vomiting	T	Diarrhoea	U	Conjunctivitis	V	Others	W	Pharyngitis	X	Follicular tonsillitis	Y	Others	M	β. haemolytic streptococcal
I 122	F 4	+		+		+		+		-		+		+		+		-		-		-		-		-		-	
I 139	M 6	+		+		-		+		-		+		+		-		-		-				-		-		Group A Type 28	
I 152	M 6	-		+		-		+		-		+		+		-		-		Night stomatitis				-		Clinical herpes simplex		-	
I 199	M 8	+		+		-		+		+		-		-		-		-		-		-				-		-	
I 155	F 9	+		+		-		+		-		+		-		-		-		-				-		-		-	
I 239	F 6	+		+		-		+		-		-		-		-		-		-		-				-		-	
I 301	F 6	+		+		+		+		-		-		-		-		-		-		-				-		-	
Totals	Males 7 Females 8	12		14		5		14		3		10		0		0		0		4		8		7		0		2	

Index No.	Age and Sex	Mo	Sore Throat	N	Pain on swallowing	O	Tonsillar exudate	P	Cervical adenopathy	Q	Nasal discharge	R	Pyrexia	S	Vomiting	T	Diarrhoea	U	Conjunctivitis	V	Others	W	Pharyngitis	X	Follicular tonsillitis	Y	Others	M	β. haemolytic streptococcal
I3	M 6	-		-		+		+		+		+		-		-		-		Febrile cold		-				-		-	
I9a	F 7	+		+		+		+		+		-		-		-		-		-				-		-		-	
I28	M 8	+		+		-		+		-		+		-		-		-		-				-		-		-	
I32	M 8	+		+		-		+		-		+		-		-		-		Cold, Pain Headache		-				-		-	
I43	F 3½	+		+		-		-		-		-		-		-		-		-				-		-		-	
I44	M 10	-		+		+		+		-		+		-		-		-		Swelling in neck		-				-		-	
I79	F 8	+		+		-		+		-		+		-		-		-		-				-		-		Group A types 4 and 28	
I 111	F 12	+		+		-		+		-		+		-		-		-		-		-		-		-		-	

VIRUS TABLE I.

Herpes simplex virus from index cases.

VIRUS TABLE II.

Influenza B Viruses from Index Cases.

		PRESENTING SYMPTOMS										DIAGNOSIS			
		Mc	N	O	P	Q	R	S	T	U	V	W	X	Y	
Index No.	Age and Sex	Sore Throat	Pain on swallowing	Tonsillar exudate	Cervical adenopathy	Nasal discharge	Pyrexia	Vomiting	Diarrhoea	Conjunctivitis	Others	Pharyngitis	Follicular tonsillitis	Others	β. haemolytic streptococci
I 4	F 12	+	+	-	+	-	+	-	-	-	-	-	+	-	-
I 7	M 6	-	-	-	+	+	+	-	-	-	Rubelliform rash	-	-	Echo ?	-
I 11	M 11	+	-	-	+	-	+	-	-	-	-	+	-	-	-
I 16	M 12	+	-	-	+	-	+	-	-	-	-	+	-	-	C or G
I 17	F 11	+	+	-	+	-	+	-	-	-	-	-	+	-	-
I 19	M 5	-	-	+	+	+	-	-	-	-	Febrile convulsions	+	-	-	-
I 22	M 9	-	-	+	+	+	+	-	-	-	Headache & nausea	-	+	-	-
I 27	F 12	+	+	-	+	-	+	-	-	-	-	+	-	-	-
I 29	M 5	+	+	+	+	+	-	-	-	-	-	+	-	-	Group A 8, 25
I 35	M 8	+	+	+	-	+	-	-	-	-	-	-	+	-	Group C or G
Totals	M 7 F 3	7	5	4	9	5	7	0	0	0	3	5	4	1	3

VIRUS TABLE III

Parainfluenza 1 and 2 Viruses from Index Cases.

Index No.	Age and Sex	M Sore throat	N Pain on swallowing	O Tonsillar exudate	P Cervical adenopathy	Q Nasal discharge	R Pyrexia	S Vomiting	T Diarrhoea	U Conjunctivitis	V Others	W Pharyngitis	X Follicular tonsillitis	Y Others	Streptococcal Association
I 159 PF1	M 5	-	-	-	+	-	+	-	-	-	-		-	-	-
I 173 PF1	M 4	+	+	-	+	+	-	-	-	-	-	-		-	A type 3
I 178 PF1	M 13	-	+	-	-	-	-	-	-	-	Pain in chest	-		-	-
I 205 PF1	F 14	+	-	-	-	+	+	-	-	-	-		-	-	-
I 181 PF2	M 14	+	+	-	-	-	-	+	-	-	-	-	-	Resp. Tract infection	-
I 275 PF2	M 10	+	+	-	+	+	+	-	-	+	Hoarse voice		-	Conjunctivitis	-
TOTALS	Males 5 Females 1	4	4	-	3	3	3	1	-	1	2	3	2	2	1

PF1 = parainfluenza 1 PF2 = Parainfluenza 2

VIRUS TABLE IV

Rhinovirus from Index Cases.

		PRESENTING SYMPTOMS										DIAGNOSIS			
		Mc	N	O	P	Q	R	S	T	U	V	W	X	Y	
Index No.	Age and Sex	Sore throat	Pain on swallowing	Tonsillar exudate	Cervical adenopathy	Nasal discharge	Pyrexia	Vomiting	Diarrhoea	Conjunctivitis	Others	Pharyngitis	Follicular tonsillitis	Others	Streptococcal Association
I 6_M	F 14	+	+	-	+	-	+	-	-	-	-	+	-	-	Group A Types 9, 18
I 20_M	M 11	-	+	-	-	-	+	-	-	-	Headache tonsils inflamed	-	-	Adenoidal fever	-
I 186_M	M 7	+	+	-	+	+	-	-	-	-	-	+	-	-	-
I 200_H	F 10	-	+	-	-	+	-	-	-	-	Acute otitis media left	-	-	Laryngitis	-
I 220_M	M 11	+	+	-	+	-	+	-	-	-	Erythema nodosum	+	-	-	Group A Untyped
Totals	Males 2 Females 3	3	5	-	3	2	3	-	-	-	3	3	-	2	

VIRUS TABLE V.

Adenovirus from Index Cases

		PRESENTING SYMPTOMS										DIAGNOSIS			
Index No.	Age and Sex	Sore throat	Pain on swallowing	Tonsillar exudate	Cervical adenopathy	Nasal discharge	Pyrexia	Vomiting	Diarrhoea	Conjuncti-vitis	Others	Pharyngitis	Follicular tonsillitis	Others	H. strepto-cocci
I 95 Adeno 1	0-3 M	NO DETAILS AVAILABLE													
I 276 Adeno 3	7 M	+	+	-	+	-	+	-	-	-	-	+	-	-	-
Totals	Male 2	1	1	0	1	0	1	-	-	-	-	2	-	-	-

VIRUS TABLE VI.

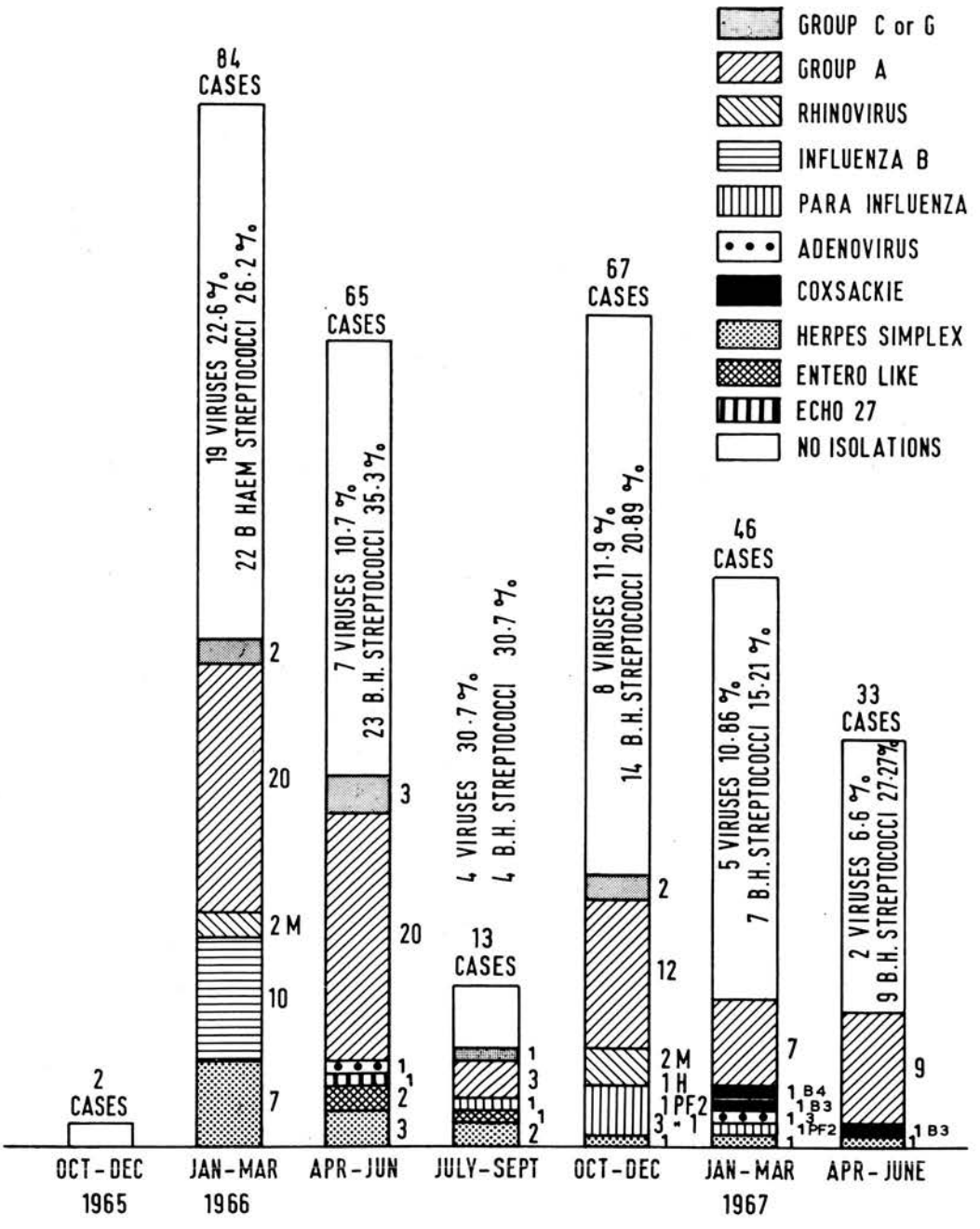
Enterovirus isolated from the Index Cases

Index No.	Age and Sex	Mc	N	O	P	Q	R	S	T	U	V	W	X	Y	
		Sore throat	Pain on swallowing	Tonsillar exudate	Cervical adenopathy	Nasal discharge	Pyrexia	Vomiting	Diarrhoea	Conjuncti-vitis	Others	Pharyngitis	Follicular tonsillitis	Others	Streptococci
I 133 Echo 27	M 15	+	+	+	+	-	+	-	-	-	-	-		-	-
I 135 Enterolike	M 6	+	-	+	+	-	+	-	-	-	-	-		-	C or G
I 137 Enterolike	F 8	+	+	-	+	-	+	-	-	-	-		-	-	
I 154 Enterolike	F 9	+	+	-	+	-	+	-	-	-	-		-	-	-
I 270 Cox B_4	M 6	+	+	-	+	-	+	-	-	-	Sore head		-	-	
I 277 Cox B_3	F 4	+	+	+	+	-	+	-	-	-	-	-		-	
I 285 Cox B_3	M 15	+	+	+	+	-	+	-	-	-	-	-		-	
Totals	Males 4 Females 3	7	6	4	7	-	7	-	-	-	1	3	4	-	1

HISTOGRAMS.

HISTOGRAM 1

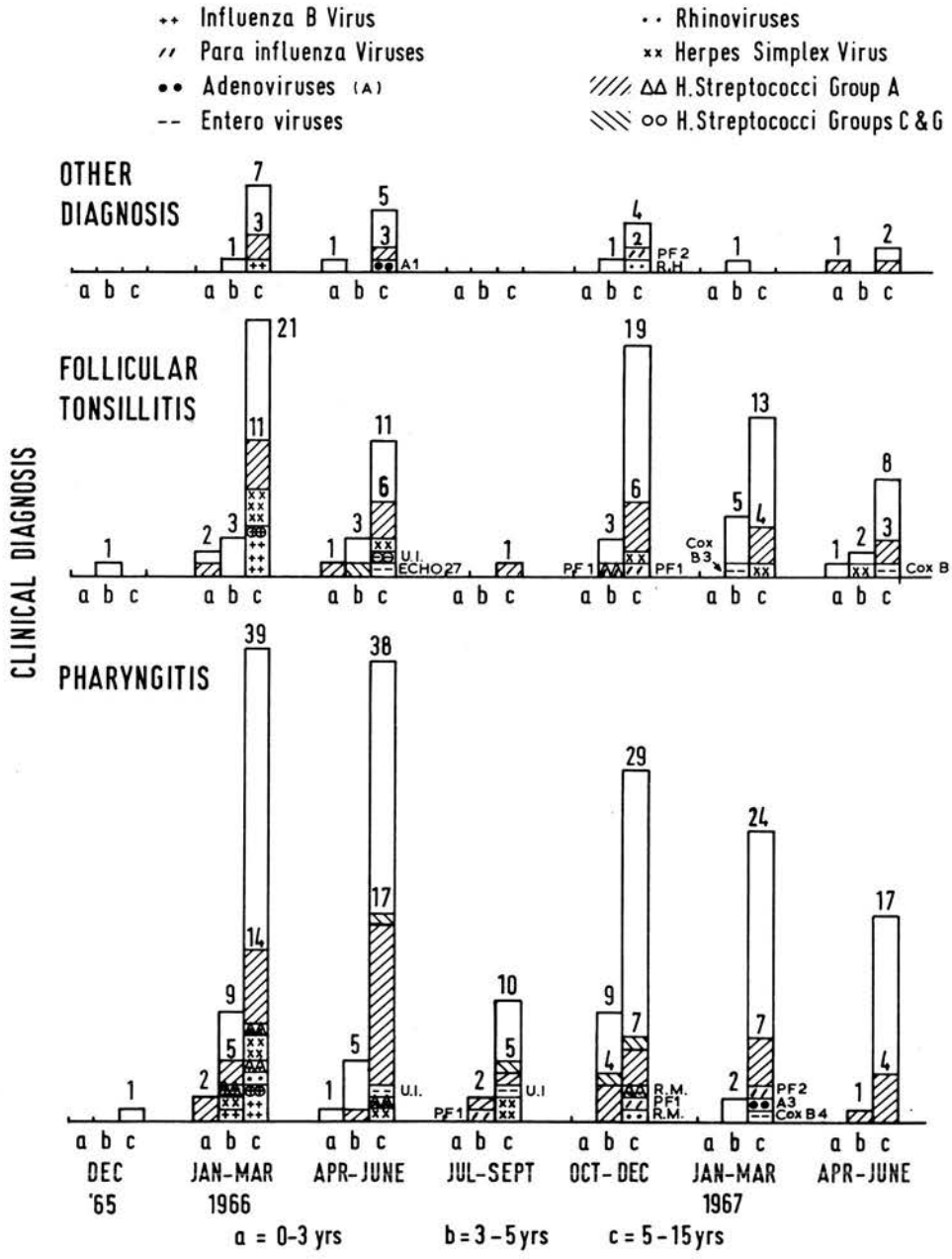
SEASONAL ISOLATION OF VIRUSES AND B HAEMOLYTIC STREPTOCOCCI FROM INDEX CASES



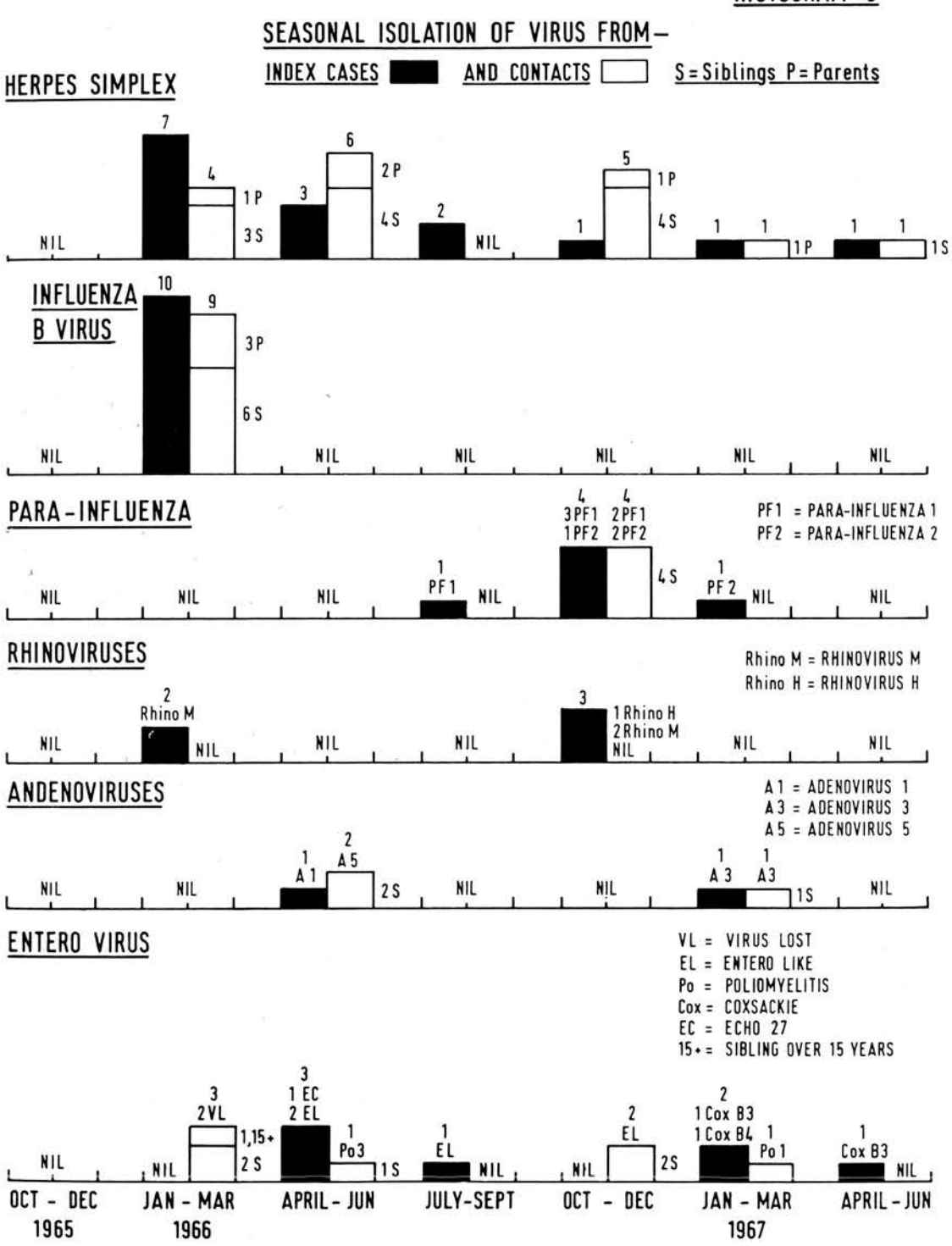
HISTOGRAM 1

HISTOGRAM 2

Nº of Index cases with their age ditribution diagnosis, virological findings and streptococcal isolations thoughout the study.

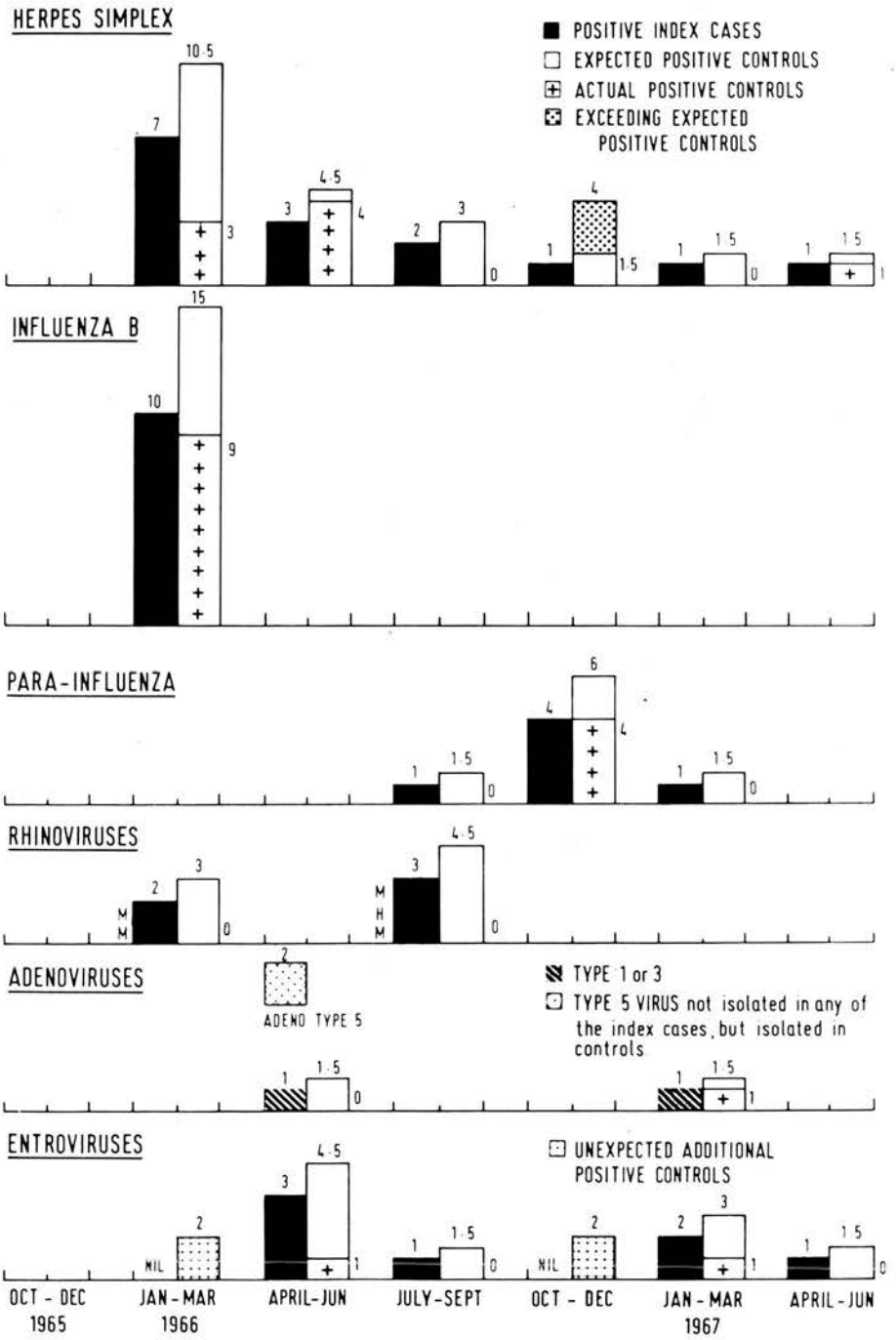


HISTOGRAM 3


SEASONAL ISOLATION OF VIRUS FROM—
INDEX CASES
AND CONTACTS
S = Siblings P = Parents
HERPES SIMPLEX
NIL
7
4
1 P
3 S
3
6
2 P
4 S
2
NIL
1
5
1 P
4 S
1
1
1 P
1
1
1 S
INFLUENZA B VIRUS
10
9
3 P
6 S
NIL
NIL
NIL
NIL
NIL
NIL
PARA-INFLUENZA
PF1 = PARA-INFLUENZA 1
PF2 = PARA-INFLUENZA 2
NIL
NIL
NIL
1 PF1
NIL
4 3PF1 1PF2
4 2PF1 2PF2
4 S
1 PF 2
NIL
NIL
RHINOVIRUSES
Rhino M = RHINOVIRUS M
Rhino H = RHINOVIRUS H
NIL
2 Rhino M
NIL
NIL
NIL
3
1 Rhino H
2 Rhino M
NIL
NIL
NIL
ANDENOVIRUSES
A 1 = ADENOVIRUS 1
A 3 = ADENOVIRUS 3
A 5 = ADENOVIRUS 5
NIL
NIL
1 A 1
2 A 5
2 S
NIL
NIL
1 A 3
1 A3
1 S
NIL
ENTERO VIRUS
VL = VIRUS LOST
EL = ENTERO LIKE
Po = POLIOMYELITIS
Cox = COXSACKIE
EC = ECHO 27
15+ = SIBLING OVER 15 YEARS
NIL
NIL
3 2VL
1,15+ 2 S
3 1 EC 2 EL
1 Po3
1 S
1 EL
NIL
NIL
2 EL
2 S
2 1 Cox B3 1 Cox B4
1 Po 1
1 Cox B3
NIL
OCT - DEC 1965
JAN - MAR 1966
APRIL - JUN
JULY-SEPT
OCT - DEC
JAN - MAR 1967
APRIL - JUN

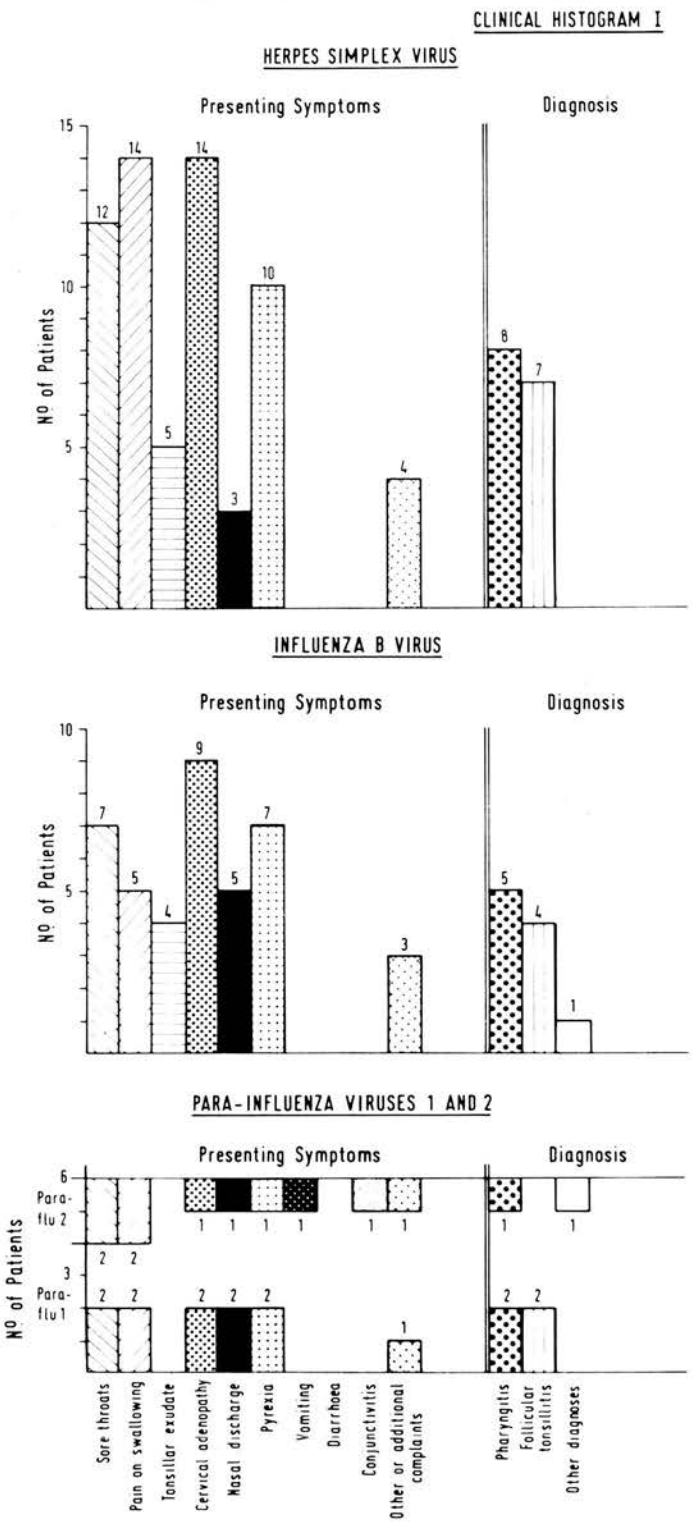
HISTOGRAM 4

DIFFERENCES BETWEEN EXPECTED AND ACTUAL ISOLATIONS OF VIRUSES IN CONTROLS BETWEEN 0 - 15 YRS.



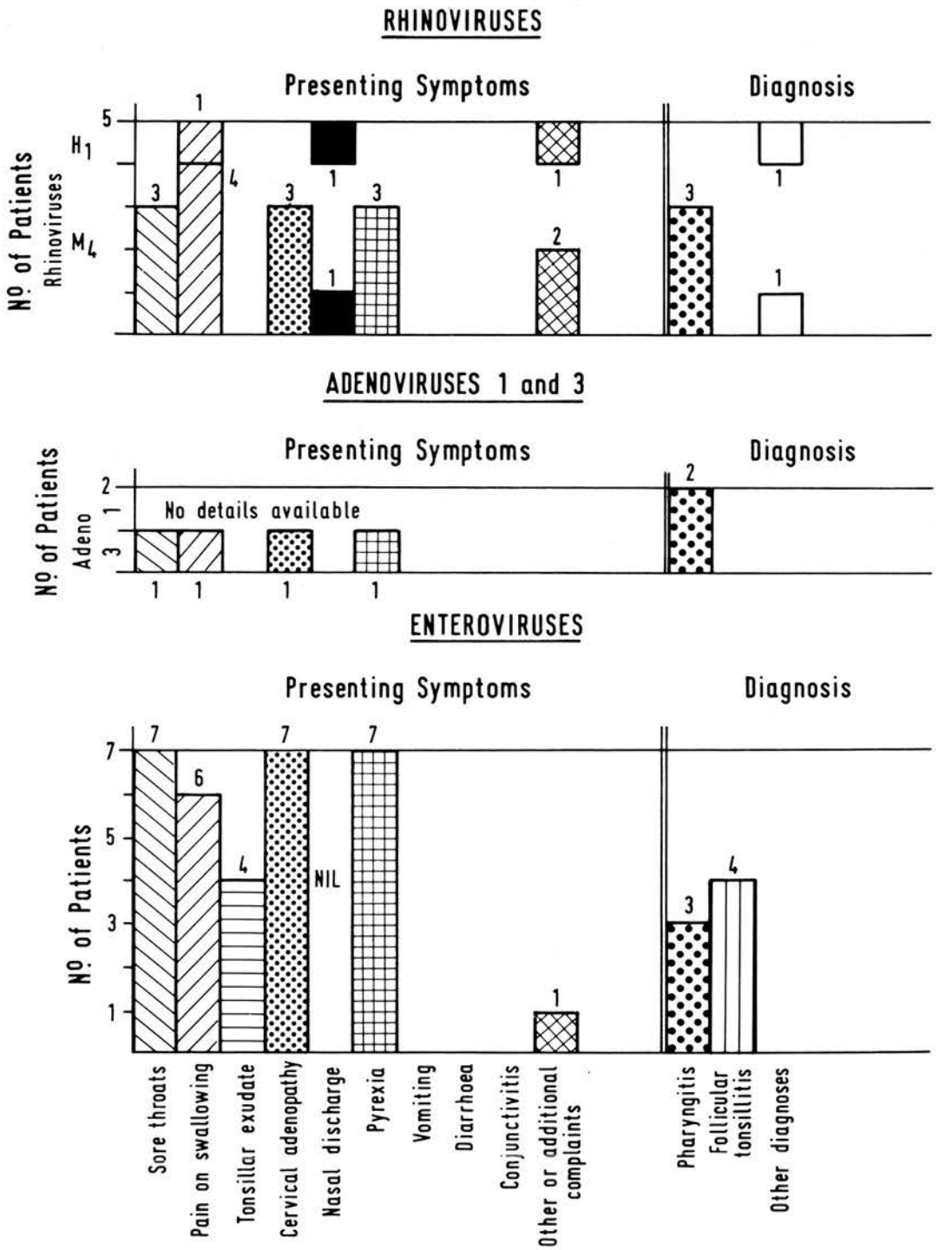
HISTOGRAM 4

Clinical Histograms.



CLINICAL HISTOGRAM I

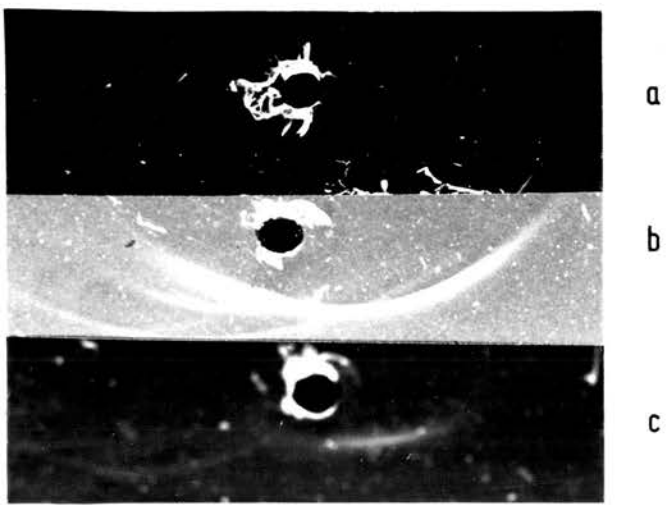
CLINICAL HISTOGRAM II



FIGURES

1A, 1B, 2A, 2B, 3A and 3B.

Rabbit 1. Pre-inoculation.



a Eluted from filter paper. CFT titre A<16 B<16

b Serum dialyzed only. CFT titre A 32 B 16

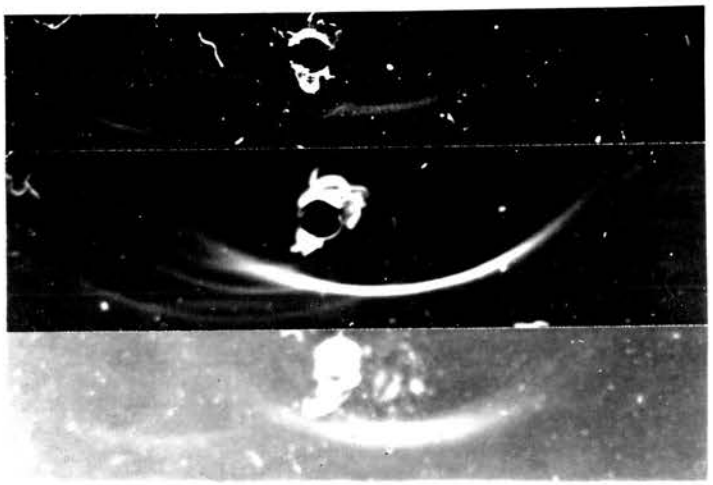
c Serum plus Mercapto ethanol. CFT titre A 16 B 16

Dilution used in test 1 : 16

FIGURE 1A.

Rabbit 1. Bled after 92 days

(Intradermal influenza vacc. + ADJ on day 1 and day 80)



a Eluted from filter paper. CFT titre A 128 B 64

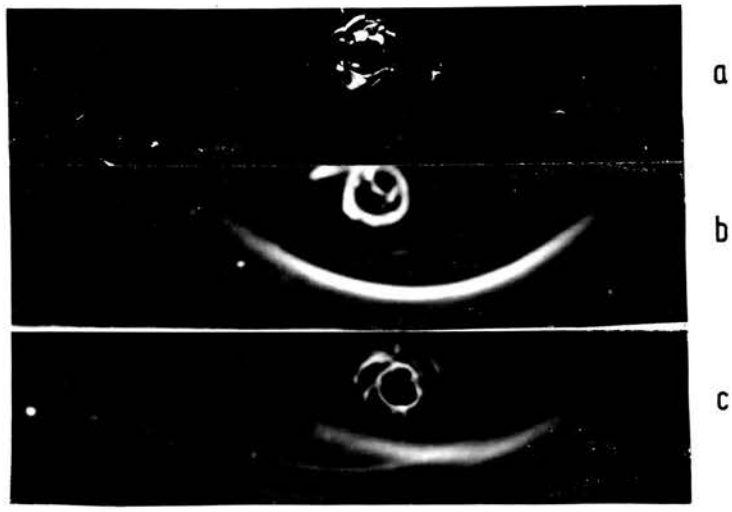
b Serum dialyzed only. CFT titre A 512 B 128

c Serum plus Mercapto ethanol. CFT titre A 16 B 32

Dilution used in test 1 : 16

FIGURE 1B.

Rabbit 4. Pre-inoculation.



a Eluted from filter paper. CFT titre A 64 B 64

b Serum dialyzed only. CFT titre A 128 B 64

c Serum plus Mercapto ethanol. CFT titre A 128 B 64

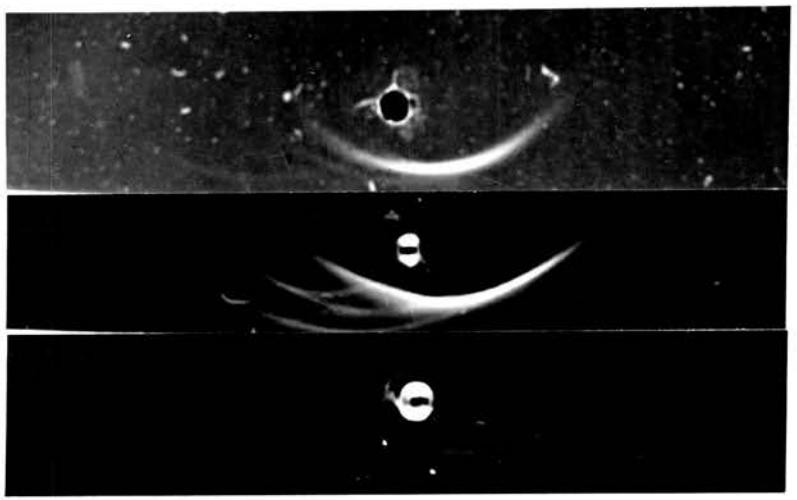
Dilutions used in test 1 : 16

FIGURE 2A.

FIGURE 2B.

Rabbit 4. Bled after 88 days

(Single inoculation of influenza vacc. on the 1st day)



a

b

c

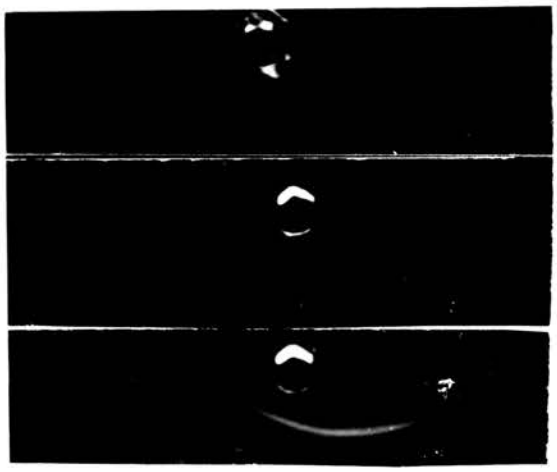
a. Eluted from filter paper. CFT titre A 128 B 128

b. Serum dialyzed only. CFT titre A 128 B 64

c. Serum plus Mercapto ethanol. CFT titre A 64 B 16

Dilutions used in test 1:16

Rabbit 5. Pre-inoculation



a Eluted from filter paper. CFT titre 16

b Serum dialyzed only. CFT titre 32

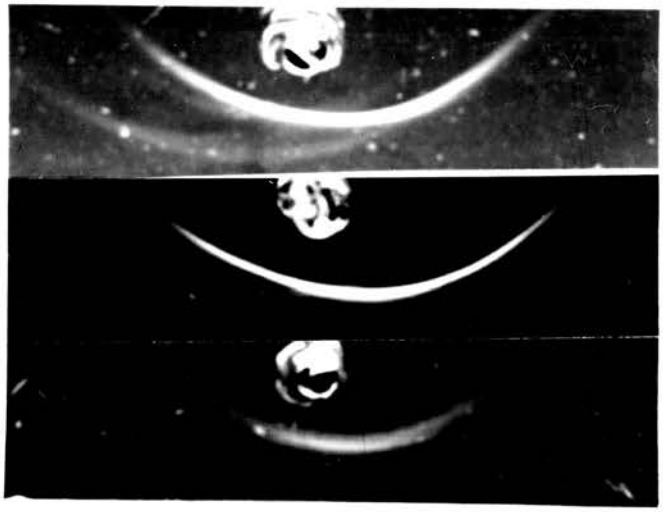
c Serum plus Mercapto ethanol. CFT titre 16

Dilutions used in test 1:16

FIGURE 3A.

Rabbit 5. Bled after 92 days

Intracorneal inoculation with Herpes Simplex Virus



a

b

c

a Eluted from filter paper. CFT titre 128

b Serum dialyzed only. CFT titre 512

c Serum plus Mercapto ethanol. CFT titre 256

Dilutions used in test 1:16

FIGURE 3B.